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Review article

Nanotechnology approaches to drug delivery for the treatment of ischemic stroke

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ABSTRACT

Ischemic stroke is a major global public health concern that lacks effective treatment options. A significant challenge lies in delivering therapeutic agents to the brain due to the restrictive nature of the blood-brain barrier (BBB). The BBB's selectivity hampers the delivery of therapeutically relevant quantities of agents to the brain, resulting in a lack of FDA-approved pharmacotherapies for stroke. In this article, we review therapeutic agents that have been evaluated in clinical trials or are currently undergoing clinical trials. Subsequently, we survey strategies for synthesizing and engineering nanoparticles (NPs) for drug delivery to the ischemic brain. We then provide insights into the potential clinical translation of nanomedicine, offering a perspective on its transformative role in advancing stroke treatment strategies. In summary, existing literature suggests that drug delivery represents a major barrier for clinical translation of stroke pharmacotherapies. While nanotechnology has shown significant promise in addressing this challenge, further advancements aimed at improving delivery efficiency and simplifying formulations are necessary for successful clinical translation.

1. Introduction

Stroke is a significant global public health issue accounting for one in every 20 deaths [1]. Stroke can be classified as ischemic, resulting from an artery blockage, or hemorrhagic, stemming from a ruptured vessel. Ischemic stroke represents the more prevalent form, constituting around 87 % of stroke cases [2]. As the population ages, the burden of ischemic stroke is predicted to rise [3]. Despite its prevalence, there are no FDA-approved pharmacotherapies for this disease.

At present, the only approved therapeutic approaches for the clinical management of stroke include intravenous tissue-type plasminogen activator (tPA) administration and mechanical thrombectomy, both designed for reperfusion [4–6]. These approaches are inherently limited in that, in addition to increasing the risk of inducing cerebral hemorrhage and secondary damage to ischemic tissue, the therapeutic window for intravenous thrombolysis is narrow [7]. Considering this time constraint and the reality that there still are no optimized reperfusion or neuroprotective strategies, the current therapies extend their benefits to only a fraction of stroke patients due to eligibility constraints.

From a pathophysiological perspective, ischemic stroke manifests as an acute deprivation of oxygen and nutrients, culminating in local acidosis, activation of the Na+/H+ exchange pathway, and a rapid surge in reactive oxygen species (ROS) [8,9]. The distorted activation of ion exchangers such as the Na+/H+ exchange pathway contributes to cellular swelling, cerebral edema, and the release of excitatory neurotransmitters, particularly glutamate. This cascade triggers excitotoxicity, initiating irreversible neuronal cell death [10] (summary of these processes seen in Fig. 1A). The dramatically increased ROS production ultimately overwhelms endogenous antioxidant mechanisms, inducing substantial toxicity to the cells within the neurovascular unit (NVU)

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[11]. In the subacute phase, inflammation initiates and is the principal driver of cell death within the ischemic brain [9]. This intricate sequence of events leads to the irreversible damage of the ischemic core, juxtaposed with the potentially salvageable hypoperfused tissue surrounding this core, known as the penumbra. An overview of the time-dependent factors of the described ischemic injury pathology can be seen in Fig. 1B. From a clinical perspective, the penumbra represents the pivotal target for managing ischemic stroke.

Due to its unique pathophysiological mechanisms, efficacious management of ischemic stroke may necessitate the formulation of multimodal therapeutic strategies aimed at precisely targeting the penumbra across distinct stages of the disease, with the imperative of timely intervention. Unfortunately, the efficient delivery of therapeutic agents to the brain poses a formidable challenge in ischemic stroke treatment, primarily due to the highly selective nature of the blood-brain barrier (BBB). Notwithstanding the potential for partial BBB compromise following ischemic insult, this barrier effectively prevents the delivery of a pharmacological dosage of most therapeutics to the brain. In this article, we undertake an in-depth review of the therapeutic agents that have been or are currently being evaluated in clinical trials, following the advancements in the development of nanotechnology approaches for drug delivery to the ischemic brain.

2. BBB as the major hurdle for stroke drug delivery

2.1. BBB and its normal physiological functions

The BBB, a selective barrier that protects the microenvironment of the central nervous system (CNS) from the peripheral circulatory system, is composed of endothelial cells (ECs), pericytes, and astrocytes. ECs line the lumen of the BBB and are situated on the apical surface of blood vessels. Unlike in other organs, ECs in the brain lack transport mechanisms and don't allow the passage of large molecules. The membrane of ECs contains various ATP-binding cassette (ABC) transporters, including P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance proteins (BCRRs), that transport selected substances that leak through ECs back into the bloodstream. Tight junctions (TJs) between ECs are formed by proteins like claudins, occludin, and junctional adhesion molecules (JAMs). These are connected to actin by scaffolding proteins known as zonula occludens (ZO) −1/2/3. Astrocytes, covering more than 90 % of the capillary walls and situated on the BBB's basal luminal membrane, provide additional support to endothelial capillaries. Astrocytes influence the BBB by regulating EC proliferation, differentiation, and the synthesis of TJs. Pericytes, positioned between ECs and astrocytes and mostly within the basement membrane, also play an important role. Pericytes contain specialized proteins like alpha-smooth muscle actin (α-SMA), tropomyosin, and myosin, endowing them with contractile ability to control BBB permeability [12]. An overview of the key components that define the BBB is also illustrated in Fig. 1A.

Due to its distinct physical and cellular makeup, the BBB effectively limits the passage of most substances from the blood to the brain through both paracellular and transcellular transport mechanisms. The BBB's selectivity permits only lipid-soluble small molecules under 400 Da, which are not substrates to the ABC transporters, to cross. While this specialized barrier staunchly prevents the infiltration of pathogens and harmful compounds from the peripheral circulatory system into the CNS, it equally hinders the entry of most therapeutic agents into the brain. Consequently, addressing diseases in the brain becomes exceptionally challenging. The selectively permeable nature of the BBB stands as the primary hurdle in delivering drugs to the CNS.

2.2. BBB compromised by an ischemic insult

Following an ischemic insult, the BBB experiences a partial disruption. In murine stroke models, investigations demonstrate that this post-stroke BBB breakdown is a reflection of intricate physiological alterations occurring at both the cellular and molecular levels [13,14]. Following an ischemic insult, the impacted regions of the brain are subjected to oxygen-glucose deprivation (OGD), triggering abnormal molecular responses across various cell types. Notably, major significant molecular responses include: 1) Activation of the ROCK/MLC pathway, resulting in the phosphorylation of myosin light chain (MLC) and actin polymerization within endothelial cells (ECs) [15]; 2) Expression and

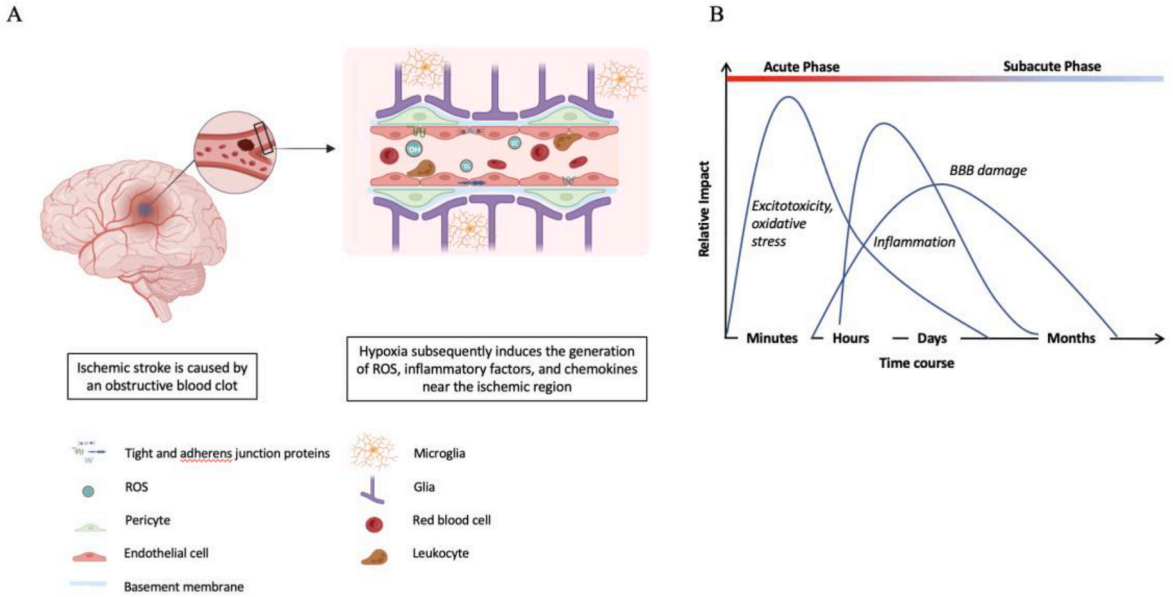


Fig. 1. An overview of the pathophysiological processes and its time course after ischemic stroke. (A) After cerebral ischemia, the resulting hypoxia triggers the activation of microglia, astrocytes, pericytes, and peripheral infiltrating cells near the ischemic site. This cascade leads to the generation of ROS, inflammatory factors, and chemokines, and, consequently, structural transformations within endothelial cells (ECs) and tight junctions (TJs). The intricate interplay between these components simultaneously contributes to the disruption and repair of the BBB. (B) The 2 weeks following ischemic insult, termed the acute phase, excitotoxicity, and oxidative stress significantly contribute to brain damage. Transitioning into the subsequent subacute phase, which can extend up to 6 months, inflammation plays a dualistic role, manifesting as both detrimental and beneficial, depending on the context. Created with BioRender.com.

activation of primary and secondary active transporters, including those found naturally in brain cells like aquaporin-4 (AQP4) [16], as well as those that emerge *de novo* in the brain post-stroke, such as the SUR1-TRPM4 channel [17], leading to cerebral edema [17]; 3) Cytosolic translocation of Caveolin-1 (Cav-1), subsequently resulting in the redistribution and endocytosis of Claudin-5 [18]; 4) Activation of the hypoxia-inducible factor (HIF) pathway, triggering the expression and activation of vascular endothelial growth factor (VEGF) and leading to the reduction of TJ proteins [19]; 5) Astrocytic expression of PDGF-β and other factors resulting in capillary contraction, hindrance of blood flow, and vesicular transcytosis [20]; 6) Generation of matrix metalloproteinases (MMPs) and adhesion molecules such as ICAM-1 and VCAM, contributing to the degradation of TJs and recruitment of peripheral leukocytes [21]; and 7) Release of cytokines, MMPs, and DNA, giving rise to the formation of neutrophil extracellular traps (NETs) and subsequent damage to the BBB [22,23] (Fig. 2).

The timeline of BBB disruption can be largely described through a biphasic pattern. One study suggested that the most pronounced BBB damage occurs at 8 h and 120 h following cerebral ischemia [24], while another study indicated that the BBB opened significantly 1.5–2 min after ischemia-reperfusion (IR), closed for 1–4 h, and then reopened after 22 h [25]. This variance between these studies likely stems from a difference in the animal models used and methodologies employed to assess BBB permeability. In human patients, it was observed that BBB compromise manifests within the initial 90 h after symptom onset. BBB permeability, quantified as the ratio of infarct permeability to contralateral permeability, was calculated to reach its peak around 6–48 h [26]. The average time from ischemia onset to the detection of BBB disruption in human patients is approximately 12.9 h [27,28]. The initiation and duration of BBB leakage due to secondary injury can span from 3 days post-stroke and may remain detectable for up to 6 months in rodents and 12 months in patients [29]. The compromised BBB is beneficial, in that it enables the infiltration of peripheral immune cells to facilitate debris clearance and wound healing, and can be seen as an opportunity to deliver therapeutics to the brain [30]. However, significantly increased BBB permeability can also be harmful, leading to neuronal dysfunction, heightened intracranial pressure, and immune-mediated damage to neurons.

3. Pharmacotherapy for ischemic stroke

Here we focus on reviewing pharmacotherapeutic agents that are designed to promote post-stroke neurological recovery. These agents are developed to mainly target detrimental factors that contribute to secondary injury, including cerebral edema, excitotoxicity, oxidative stress, and inflammation [31]. Up to the present time, over 114 clinical trials, involving 49 pharmacotherapeutic agents, have been conducted. Among them, 21 agents have undergone assessment in phase 2/3 clinical trials (Table 1). Unfortunately, the collective outcomes have proven to be less than satisfactory, with only a handful of agents exhibiting promising clinical potential.

AIS, acute ischemic stroke; ALIAS, Albumin in Acute Ischemic Stroke Trial; AXIS 2, AX200 for the Treatment of Ischemic Stroke; G-CSF, Granulocyte colony-stimulating factor; PIMSS, Perth IV Minocycline Stroke Study; CERE-LYSE-1, Combined Treatment With Alteplase (Rt-PA) and Cerebrolysin in Acute Ischemic Hemispheric Stroke; Urico-Ictus, Efficacy Study of Combined Treatment With Uric Acid and rtPA in Acute Ischemic Stroke; FAST-MAG, Field Administration of Stroke Therapy-Magnesium Trial; MASTERS, Study to Examine the Effects of MultiStem in Ischemic Stroke; MEXIDOL, ethylmethylhydroxypyridine

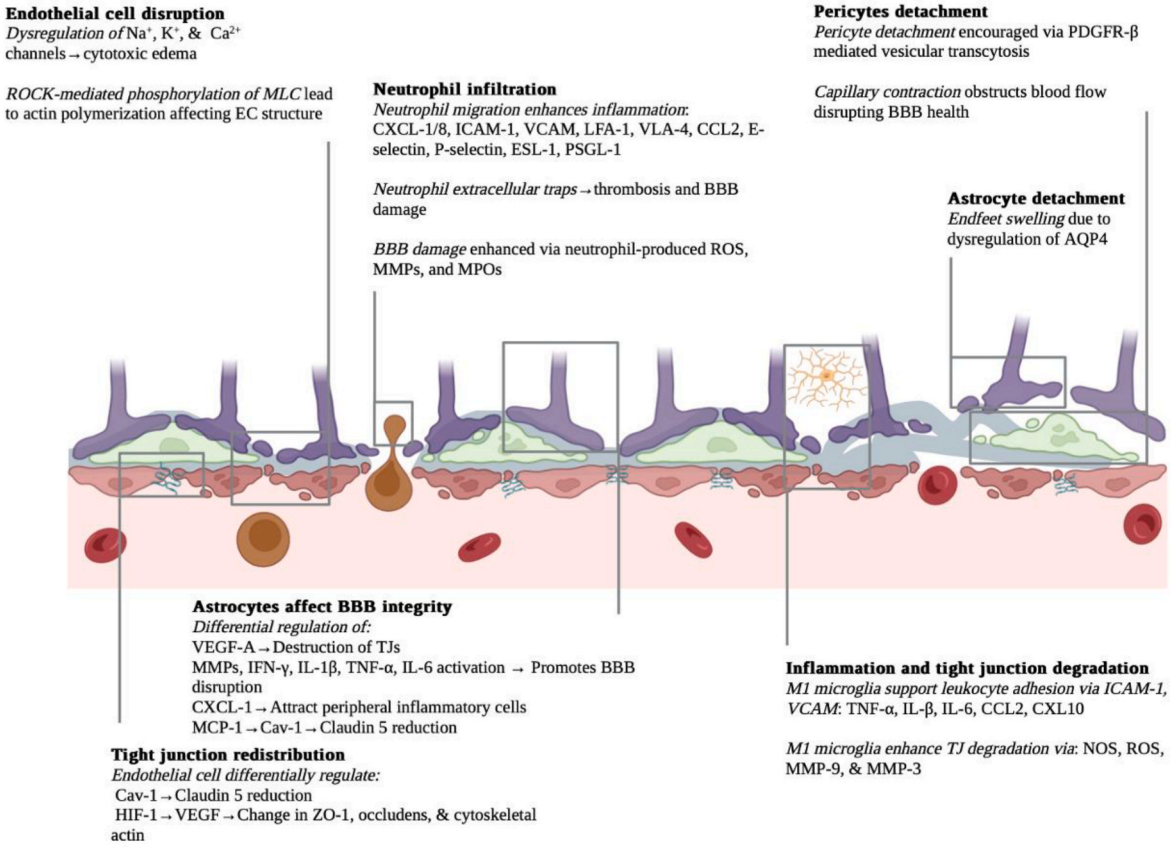


Fig. 2. Cellular and molecular mechanisms of BBB disruption after an ischemic stroke. Stroke onset initiates a heterogeneous cascade of cellular and molecular signaling pathways which result in alterations of cell structures, cell death, vasogenic and cytotoxic edema, inflammation, tight junction redistribution, and degradation, characterizing BBB disruption. Created with BioRender.com.

Table 1
Phase 2/3 clinical trial explored pharmacotherapies for ischemic stroke.

Registration number	Phase	Therapeutic	Description/Mechanism	Results	Year
ALIAS (NCT00235495)	3	Albumin	An endogenous plasma protein with important physiochemical properties, has commonly been regarded as an alternative hemodiluting agent to dextran	No clinical benefit of 25 % albumin given within 5 h after AIS	2013 [32]
AXIS 2 (NCT00927836)	2b	G-CSF	A potent neuronal growth factor with multimodal antiapoptotic, arteriogenic, and neurogenic properties	G-CSF did not provide any significant benefit with respect to either clinical outcome or imaging biomarkers	2013 [33]
PIMSS (ACTRN12612000237886)	2	Minocycline	A semisynthetic tetracycline Neuroprotection	Intravenous minocycline was safe but not efficacious	2013 [34]
CERE-LYSE-1 (NCT00840671)	3	Cerebrolysin	A low-molecular-weight neuropeptide and free amino acid of porcine origin; Neuroprotective and neurotrophic properties	The combination of Cerebrolysin with tPA is safe for treatment of acute ischemic stroke but did not improve outcome at day 90	2013 [35]
URICO-ICTUS (NCT00860366)	2b/3	Uric acid	A small molecule that has antioxidant and neuroprotectant activities	The addition of uric acid to thrombolytic therapy did not increase the proportion of patients who achieved excellent outcome after stroke compared with placebo, but it did not lead to any safety concerns	2014 [36]
NCT02002390	2	Fingolimod	A sphingosine analog that acts on sphingosine-1-phosphate receptors; Immunology modulator	Combination therapy of fingolimod and alteplase was well tolerated, attenuated reperfusion injury, and improved clinical outcomes in patients with acute ischemic stroke	2015 [37]
ISRCTN71371114	2	Erythropoietin	Anti-ischemic and anti-apoptotic properties, promotion of neovascularization, mobilization of endothelial progenitor cells, and enhancement of angiogenesis	Erythropoietin therapy significantly improved long-term neurological outcomes in patients after AIS	2015 [38]
GAMES-RP (NCT01794182)	2	Glyburide (RP-1127)	A SUR1-TRPM4 channel antagonist that reduces cerebral edema	Intravenous glyburide was well tolerated in patients with large hemispheric stroke at risk for cerebral edema. There was no difference in the composite primary outcome	2016 [39]
FAST-MAG (NCT00059332)	3	Magnesium	Vasodilatory and neuroprotective & glioprotective effects	Magnesium is not biologically neuroprotective in acute stroke	2017 [40]
MASTERS (NCT01436487)	2	Multipotent adult progenitor cells	A bone marrow-derived, allogeneic, cell therapy product modulates the immune system	Administration of multipotent adult progenitor cells was safe in patients with AIS. No significant improvement was observed at 90 days in neurological outcomes	2017 [41]
EPICA (NCT02793687)	3	MEXIDOL	A succinate salt that has antioxidant and antihypoxant and suppresses excitotoxicity	Use of Mexidol in the acute and early recovery phases of AIS is recommended	2018 [42]
ACTION II (NCT02730455)	2	Natalizumab	An α-4 integrin-targeting immunomodulation antibody that inhibits transmigration of leukocytes across the vascular endothelium	Natalizumab administered ≤24 h after AIS did not improve patient outcomes	2020 [43]
RESTORE BRAIN (NCT02877615)	2	S44819	A GABA α5 antagonist improves neurological recovery	There was no evidence that S44819 improved clinical outcome in patients after AIS	2020 [44]
ESCAPE-NA1 (NCT02930018)	3	Nerinetide	Neuroprotection A PSD95-NR2B inhibitory peptide decreases excitotoxicity	Nerinetide did not improve the proportion of patients achieving good clinical outcomes after endovascular thrombectomy. Among patients who were not treated with alteplase, they observed a treatment effect	2020 [45]
NCT02430350	3	Compound Edaravone Injection	A novel neuroprotective agent with synergistic effects of antioxidant and anti-inflammatory	When edaravone dexborneol versus edaravone was administered within 48 h after AIS, 90-day good functional outcomes favored the edaravone dexborneol group, especially in female patients	2021 [46]
CHARM (NCT02864953)	3	Glyburide (BIIB093)	Anti-edema A SUR1-TRPM4 channel antagonist reduces cerebral edema	CHARM will include 680 participants across 22 countries	Recently Completed
MASTERS-2 (NCT03545607)	3	MultiStem	Neuroprotection/immunomodulation Adult-derived multipotent adult progenitor cells to provide neuroprotection, immunomodulation, and (less likely) cell replacement effects	300 participants; Single intravenous infusion 18–36 h after AIS	Ongoing
PMZ-1620 (NCT04047563)	3	Sovateltide (PMZ-1620)	Neurological recovery An endothelin-B receptor agonist augments the activity of neuronal progenitor cells	110 participants; Efficacy of sovateltide (PMZ-1620) in patients of AIS	Ongoing
NCT03639922	3	Imatinib	Reduces intracerebral hemorrhage and edema	1260 participants; Imatinib in acute ischemic stroke	Ongoing
NCT04904341	3	Cerebrolysin	A low-molecular-weight neuropeptide and free amino acid of porcine origin; Neuroprotective and neurotrophic properties	50 participants; Efficacy of cerebrolysin treatment as an add-on therapy to mechanical thrombectomy in AIS	Ongoing

succinate; ACTION II, Safety and Efficacy of Intravenous Natalizumab in Acute Ischemic Stroke; RESTORE BRAIN, Efficacy and Safety Trial With S 44819 After Recent Ischemic Cerebral Event; ESCAPE-NA1, Safety and Efficacy of Nerinetide (NA-1) in Subjects Undergoing Endovascular Thrombectomy for Stroke; CHARM, Phase 3 Study to Evaluate the Efficacy and Safety of Intravenous BIIB093 (Glyburide) for Severe Cerebral Edema Following Large Hemispheric Infarction; MASTERS-2, MultiStem® Administration for Stroke Treatment and Enhanced Recovery Study; PMZ-1620, Efficacy of Sovateltide (PMZ-1620) in Patients of Acute Ischemic Stroke.

3.1. Glibenclamide

Cerebral edema commonly develops in patients with moderate to severe stroke, denoted by an NIH Stroke Scale (NIHSS) score exceeding 4, accounting for around half of the U.S. stroke cases [47,48]. The progression of cerebral edema in stroke patients unfolds through three stages [49,50]. In the initial stage, cytotoxic edema emerges shortly after stroke onset. This process involves cellular swelling caused by an influx of osmolytes, primarily Na+ and Cl–, guiding the movement of water from interstitial spaces into cells. This phenomenon takes place across all brain cell types. Following cytotoxic edema, the second stage sees the formation of ionic edema. This extracellular edema occurs while the BBB remains intact [51]. The transendothelial Na + gradient from cytotoxic edema drives the extravasation of osmolytes and water, propelled by potential energy. Hours after the ischemic insult, the final stage involves partial disruption of the BBB. Transendothelial channels allow protein and water passage from the vascular to the interstitial compartment, culminating in vasogenic edema.

At the molecular level, primary and secondary active transporters drive cytotoxic and ionic edema, including those found in brain cells, such as aquaporin-4 (AQP4), and those emerging *de novo* after stroke, like the SUR1-TRPM4 channel [17]. The SUR1-TRPM4 channel, typically not expressed or expressed minimally in a normal brain, becomes active when intracellular ATP is depleted following ischemic stroke. Under cerebral ischemia and hypoxia conditions, the SUR1-TRPM4 channel's expression surges across all cells in the NVU, including capillary endothelial cells, neurons, astrocytes, and oligodendrocytes [52–55]. Abnormal SUR1 expression leads to sustained sodium influx, subsequent water influx, oncotic cell swelling, and cell death, resulting in space-occupying edema and even hemorrhagic transformation [51,53,56]. The SUR1-TRPM4 channel's activity can be effectively blocked by glyburide (INN: glibenclamide), a sulfonylurea diabetes medication that binds to SUR1 with subnanomolar affinity [57,58]. Animal studies showed that glyburide administration reduces edema, lesion volume, and mortality, and enhances neurological outcomes [53,59–63]. These pre-clinical studies led to clinical trials, where the glyburide formulation was designated as RP-1127. Initially assessed in the GAMES-PILOT study with 10 stroke patients [64,65], it was further evaluated in the GAMES-RP phase II clinical trial with 86 patients (18–80 years old) experiencing acute large-area AIS (within <10 h, average 9 h) [39]. At the 90-day follow-up, the glibenclamide group displayed no distinct primary endpoint differences compared to the placebo group. Subsequent analysis revealed reduced brain midline shift and a trend toward improved survival, implying reduced mass effect and water uptake [39,66]. These promising findings led to FDA approval for the recently completed phase III clinical trial, CHARM (NCT02864953) [67].

3.2. NA1

One of the primary mechanisms of neuronal injury following stroke is NMDAR-dependent excitotoxicity, which is induced by the binding of PSD-95 with NMDARs and nNOS at excitatory synapses [68,69]. It was shown that protection of neurons against NMDAR-mediated excitotoxicity could be achieved through disruption of the interaction of NMDARs with PSD-95 using the last nine amino acids of the carboxyl tail of GluN2B (NR2B9c) [68]. Since NR2B9c cannot penetrate the cell membrane, therapeutic use of NR2B9c requires fusion with TAT, a cell penetration peptide. The resulting Tat-NR2B9c peptide was evaluated in rodent and nonhuman primate models and demonstrated a significant reduction in infarct volume and improvements in neurological outcomes [68,70]. The promising pre-clinical studies led to serial clinical trials, in which Tat-NR2B9c was rebranded as NA1 [45, 71]. Results of the phase II clinical trial ENACT found that treatment with NA1 effectively reduced the number and volume of iatrogenic embolization strokes in patients with endovascular aneurysm repair [71]. These findings led to a multi-center, double-blind, randomized phase III clinical trial, ESCAPE-NA1, which was reportedly completed in 2020 [45]. Although the overall results of the study were neutral, NA1 treatment was found to be associated with improved 90-day clinical outcomes (59.3 % vs 49.8 %, RR 1.18, 95 % CI 1.10 to 1.38).

Further analysis showed that tPA degrades NA1, making NA1 in tPA-treated patients ineffective. To overcome this limitation, a new generation of plasmin-resistant NA1 formed by substituting cleavage-prone amino acids from their l-to their d-enantiomeric form was developed [72]. Currently, the new plasmin-resistant NA1 is being tested in human patients.

3.3. Uric acid (UA)

In response to the need for neuroprotective strategies alongside mechanical thrombectomy, the National Institutes of Health (NIH) recently launched the Stroke Preclinical Assessment Network (SPAN). This initiative was designed to evaluate selected pharmacotherapies for the treatment of ischemic stroke. In a recently concluded phase 1 pilot study, uric acid (UA) emerged as a particularly promising neuroprotective candidate based on its performance in rodent models [73]. UA, a product of purine metabolism, is typically quickly degraded by hepatic uricase to allantoin in most mammals. However, due to the nonfunctional uricase gene in humans [74], UA levels tend to be higher in humans than in most mammals.

UA acts as a robust endogenous antioxidant and scavenger of free radicals. Notably, its levels can surge during periods of high oxidative stress, such as in the case of ischemic stroke. Earlier experiments conducted on rats found that in ischemic conditions, adenosine triphosphate (ATP) degradation led to the generation of adenine and xanthine. This, in turn, triggered an upswing in xanthine oxidase production, resulting in increased UA generation and elevated oxidant formation [75].

In preclinical studies, UA has demonstrated a capacity to enhance stroke outcomes in various stroke models derived from hyperglycemic, female, and male mice [76,77]. It was also found that treatment with UA is synergistic with rtPA infusion [78]. There is evidence that UA has a limited ability to cross the BBB, as intravenous administration of UA led to increased plasma levels within 10 min, whereas brain tissue UA levels remained unaffected [79,80].

In the clinical domain, a study by Chamorro and colleagues reported a 12 % increase in the odds of a favorable clinical outcome for each milligram per deciliter of serum uric acid added [81], while another study suggested that low UA concentrations are modestly associated with positive short-term outcomes [82]. A pilot clinical study found that intravenous UA administration alongside rtPA infusion within 3 h of symptom onset was safe and that the treatment reduced the level of activated MMP-9 [83]. The Phase 2 URICO-ICTUS trial, involving 421 AIS patients treated with rtPA within 4.5 h of symptom onset, confirmed the safety of a single 90-min infusion of 1 g UA administration. Although there was an overall nonsignificant 6 % rise in the rate of favorable outcomes at follow-up, significant effects of UA therapy were found in various subgroups including women, hyperglycemic patients, and those treated with mechanical thrombectomy [84].

3.4. Minocycline

With its high lipophilicity, minocycline can penetrate the BBB. The compound's neuroprotective effects primarily stem from its capacity to inhibit microglial activation. This capacity, in turn, reduces T cell migration, cellular apoptosis, free radical generation, and inflammatory responses [85,86]. Moreover, minocycline exhibits a significant ability to restrain the production of MMP-9 [87], a pivotal factor implicated in the transformation of intracranial hemorrhage associated with thrombolytic therapy. Consequently, minocycline holds the potential for diminishing tissue damage and hemorrhage transformation. Supported by a Dose-Finding Study [88], minocycline is safe and compatible with tPA infusion. While the effectiveness of minocycline in treating acute ischemic stroke (AIS) was demonstrated in two distinct clinical trials [89,90], uncertainties persist regarding its capacity to significantly curtail mortality and disability post-AIS [34].

3.5. Other therapeutic agents

Numerous other therapeutic agents hold potential promise for the treatment of ischemic stroke. These include fasudil, a Rho-associated kinase (ROCK) inhibitor, tocilizumab (Actemra), an immunosuppressive agent, fingolimod (Gilenya, FTY720), an S1P analogue, and NEP1-40, a Nogo receptor (NgR) antagonist peptide. Among them, fasudil, tocilizumab, and fingolimod were characterized in the recently completed SPAN phase 1 pilot study.

Fasudil is a non-specific inhibitor of Rho kinases (ROCK), which are well-characterized therapeutic targets for ischemic stroke [91]. In rodent studies, Fasudil demonstrated compatibility with tPA as well as the potential to prevent MMP-9-related hemorrhagic transformation and reduce cerebral infarct size while improving neurological outcomes [92–95]. Fasudil was tested in a placebo-controlled double-blind trial for its safety and efficacy. Fasudil was administered within 48 h of ischemic stroke onset and results revealed fasudil is safe for human use and that treatment with fasudil significantly improves clinical outcomes [96].

Veliparib is an inhibitor of poly (ADP-ribose) polymerase (PARP), which plays a pivotal role in the repair of DNA damage by catalyzing the transfer of an ADP ribose unit from NAD + to specific target proteins, including histones and transcription factors [97]. When PARP becomes overly active, it disrupts mitochondrial homeostasis by depleting NAD + reserves. This depletion leads to an escalation in the levels of reactive oxygen and nitrogen species, as well as a surge in intracellular Ca2+ concentrations. Several studies have demonstrated that PARP inhibitors can prevent the activation of microglia and enhance neuronal survival [98,99]. However, the exploration of veliparib's application for treatment of ischemic stroke remains limited.

Tocilizumab is a humanized monoclonal anti-interleukin-6 receptor antibody and used as an immunosuppressive medication for treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis. As cerebral ischemia causes time-dependent recruitment and activation of inflammatory cells, inhibition of the inflammatory response has the potential to reduce ischemic size and improve neurological outcomes. Experimental studies in murine stroke models showed that treatment with tocilizumab lowered the immune system's response to stroke and that the neuroprotective dose of tocilizumab was different between males and females [100,101].

Fingolimod functions as an agonist for sphingosine 1-phosphate (S1P) receptors and has demonstrated neuroprotective effects in various animal stroke models [102,103]. Mechanistically, fingolimod acts by impeding the migration of lymphocytes during the reperfusion phase. This action leads to the attenuation of inflammation within cerebral blood vessels and neural tissue, fostering improved blood circulation and evident neuroprotective outcomes. Fingolimod has been evaluated in two small-scale trials, where it significantly reduced infarct size and ameliorated neurological deficits in stroke patients [37,104]. Furthermore, fingolimod was characterized in a randomized and blinded clinical study involving 23 stroke patients who received either tissue plasminogen activator (tPA) or a combination of tPA and fingolimod within a time frame of 4.5–6 h after stroke onset. Results showed that the combination therapy group displayed not only superior clinical enhancements compared to the tPA-alone group but also notable improvements in anterograde reperfusion and retrograde collateral blood flow [105].

NEP1-40 is a Nogo receptor (NgR) antagonist peptide, which can inhibit the effect of Nogo-A and enhance short-term neurosynaptic plasticity and neurological function after cerebral ischemia [106]. Nogo-A has the effect of resisting synapse reconstruction after CNS injury. NgR1 is the receptor of Nogo-A. Inhibiting NgR1 can promote axon regeneration. Similar to NR2B9c, NEP1-40 is also a peptide drug. Therefore, they possess similar issues with ensuring efficient delivery.

4. Nanotechnology as a potential solution to address the challenges of effective drug delivery

As surmised, the therapeutic window for many effective pharmacotherapies is within the first 12 h of stroke onset yet, this presents a challenge as the mean time from the onset of ischemia to observation of BBB disruption is around 12.9 h [27,28,107]. In this period and beyond, the injured BBB is still highly selective and can be accompanied by reperfusion injury, tissue no-reflow, poor collateral circulation, hemorrhage transformation, impaired cerebrovascular auto-regulation function, and large hypoperfusion volume, all factors that impact clinical prognosis and treatment design [108]. As such, the BBB remains a major barrier to the development of pharmacotherapies that effectively treat stroke and might account for why some pharmacotherapeutic agents showed limited efficacy in the clinic. For example, it was well characterized that glyburide has a limited ability to penetrate the brain [109,110]. Using PET/CT and labeling glyburide with a radiotracer, we demonstrated there was no significant difference in glyburide uptake between the ischemic and the contralateral hemispheres in rats bearing large stroke, suggesting that the penetrability of glyburide is not enhanced in the ischemic brain [109]. To overcome the drug delivery hurdle, emerging nanotechnology approaches hold great promise [111–114] as nanoparticles (NPs) can potentially be engineered through various mechanisms to enhance drug accumulation in the ischemic brain tissue [115–118]. Employing NPs has additional advantages in that they provide physical protection to cargo agents that otherwise are insoluble, unstable, toxic, or substrates to ATP-binding cassette (ABC) transporters on the BBB.

4.1. Applications of NPs for drug delivery to the ischemic brain

NPs can be classified based on their main compositions. Though not all types are extensively covered in this review, Fig. 3 exemplifies the structures of many NPs types and subtypes that are studied for ischemic stroke treatment. Please note this list is not exhaustive. To date, various types of NPs, including polymeric NPs, lipid NPs, small-molecule self-assembled NPs, protein NPs, metal NPs, and extracellular vesicles, have been explored for drug delivery to the ischemic brain.

4.1.1. Polymeric NPs

Polymeric NPs, mainly including solid NPs, micellular NPs, and dendrimer NPs, refer to those NPs consisting of polymeric materials, which are formed by covalent attachment of a set of small molecule monomers. This class of NPs often has excellent biocompatibility and sustained-release characteristics, making them suitable for various drug delivery applications [106,113,119–121]. In a recent study using poly (lactic-co-glycolic acid) (PLGA) as the starting material, one of the most often used polymers in the biomedical field, we synthesized and engineered solid polymeric NPs for autocatalytic brain targeting through surface conjugation of chlorotoxin and internal encapsulation

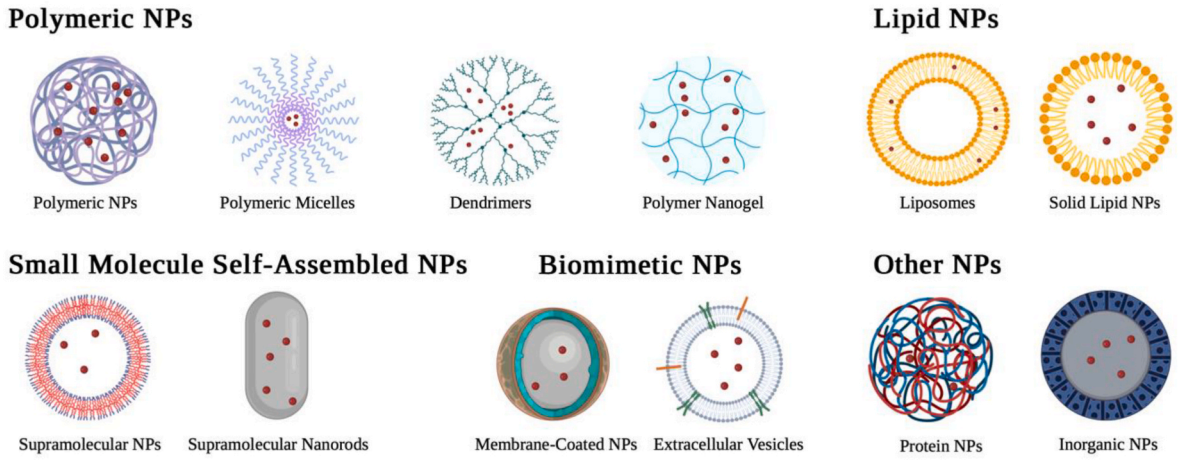


Fig. 3. Schematic representation of the types of NPs that can potentially be engineered for the treatment of ischemic stroke. Created with BioRender.com.

of lexiscan. We showed that the resulting NPs were capable of efficiently penetrating the ischemic brain and delivering peptide NEP1-40 for effective treatment of ischemic stroke [106] (Fig. 4). Through a similar approach, Lu and colleagues synthesized micellular NPs using a ROS-scavenging polymer and showed that the NPs could be utilized for efficient delivery of rapamycin to the ischemic brain for stroke treatment [122].

Unlike solid NPs, which maintain their structures during delivery, micellular NPs have a relatively unstable structure and could be engineered to respond to external stimuli by changing size. We recently synthesized polycaprolactone (PCL)-derived micellular NPs, which respond to thrombin, a protease preferentially accumulated in the ischemic microenvironment, by expanding or shrinking in size. The approach to engineering size-changing NPs will be discussed in section 4.2.2. We found that the micellular NPs were able to efficiently penetrate the ischemic brain and deliver the glyburide payload to achieve effective treatment of ischemic stroke [123].

Other types of polymeric NPs, including dendrimers, polymeric nanogels, and melanin NPs, have also been explored for drug delivery to the ischemic brain. In one study, Li et al. employed dendrimers for drug delivery to the ischemic brain [124]. Compared to polymeric solid NPs and micellular NPs, dendrimers have a unique advantage in their small size and uniform dispersion [125]. The authors engineered dendrimers for brain targeting through surface conjugation of an EC-targeting peptide COG1410. They showed that the resulting NPs penetrated the brain with high efficiency, and when salvianolic acid A, an antioxidant, was conjugated, the NPs effectively promoted stroke recovery [124]. In another study, Liu and colleagues explored NPs consisting of melanin, a biological polymer known to have scavenging activities, as an antioxidant therapeutic for stroke treatment and found that the intraventricular administration of these NPs significantly reduced infarct volume [126].

4.1.2. *Lipid NPs (LNPs)*

LNPs are nanoformulations mainly consisting of lipid materials. Liposomes, an early generation LNP, possess a unique vesicular structure formed by a lipid bilayer in the shape of a hollow sphere. The double-layer structure enables liposomes to be loaded with either hydrophilic or hydrophobic drugs. Unlike liposomes, the latest LNP formulations possess a liposome-like structure but do not necessarily have a continuous bilayer.

LNPs, particularly liposomes, are the earliest nanomedicine delivery platform to have successfully advanced to clinical applications. LNPs

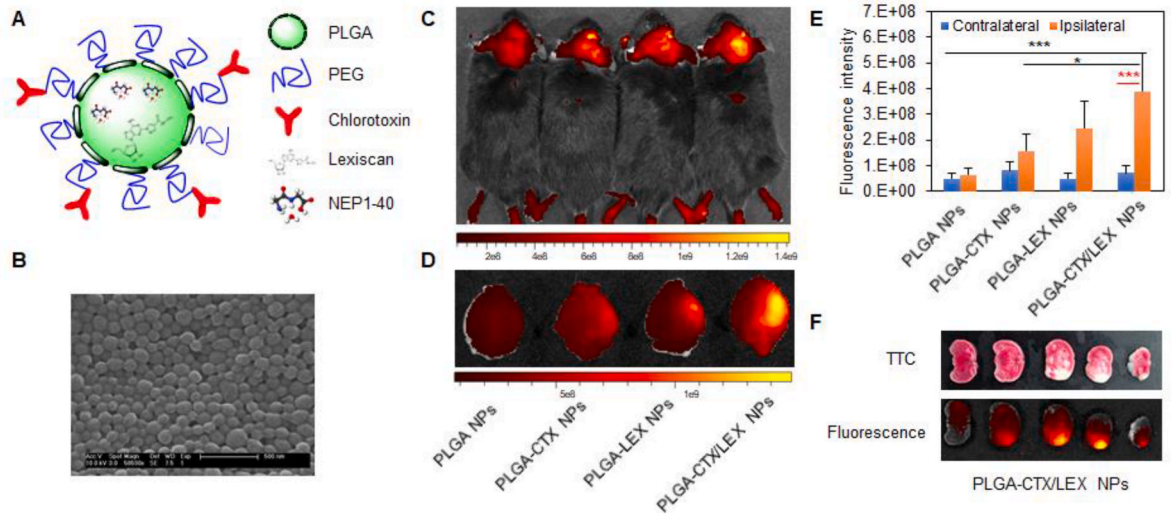


Fig. 4. Targeted drug delivery to ischemic stroke via chlorotoxin-anchored, lexiscan-loaded nanoparticles improved drug delivery capabilities. (A) Schematic diagram of a NEP1-40-loaded PLGA-CTX/LEX NP. (B) Representative image of NEP1-40-loaded PLGA-CTX/LEX NPs as captured by scanning electron microscope. Scale bar: 500 nm. (C) Representative image of NPs in the brains of live animals. (D) Representative image of NPs in the excised brains. (E) Semi-quantification of NPs in the excised brains. (F) Representative images of brain slices prepared from mice receiving 2 treatments of unlabeled PLGA-CTX/LEX NPs prior to the final administration of IR780-loaded PLGA-CTX/LEX NPs. Reproduced from Ref. [106].

have been explored for drug delivery in cases of stroke. Several studies reported that liposomes could be employed for the encapsulation of hemoglobin to deliver oxygen to ischemic brain tissue, leading to accelerated stroke recovery [127–129]. In another study, Zhao and colleagues reported the use of liposomes to deliver basic fibroblast growth factor (bFGF) to the brain and demonstrated that intranasal administration of bFGF-loaded liposomes efficiently bypassed the BBB, effectively reduced infarct volume, and promoted functional recovery [130] (Fig. 5).

4.1.3. Small molecule-assembled nanoparticles

Recently, we and others identified a group of small molecules, which bear ring structures and are capable of self-assembly into spherical and rod-shaped NPs [131–135]. Many of these molecules are unique in that they possess pharmacological activities and are derived from natural herbal materials known to be effective for stroke treatment [133]. As such, NPs consisting of these small molecules could be potentially employed not only for drug delivery to the ischemic brain but also as a therapeutic agent for stroke treatment. In a recent study [133], we studied betulinic acid (BA) and found it formed rod-shaped NPs of different sizes and shapes, including lengths of ~156 nm and diameter of ~45 nm (156(l) x 45(d)), 315(l) x 60(d), and 730(l) x 35(d) that were designated as R150, R300, and R700, respectively (Fig. 6A). We characterized all of these NPs for drug delivery to the ischemic brain and found that, after intravenous administration, R300 demonstrated the greatest efficiency (Fig. 6B and C). We showed that the excellent brain penetration capabilities of R300 can be attributed not only to their unique size and shape, but also their interaction with cannabinoid receptor 1 (CB1), which is highly expressed in ischemic brain tissue after stroke, and pre-treatment with SR141716A, a CB1 receptor blocker, reduced their brain penetrating efficiency (Fig. 6D–G). We further characterized the use of BA NPs as a drug carrier for the delivery of glyburide and found that glyburide-loaded BA NPs limit the risk of hypoglycemia induced by glyburide while achieving anti-edema and antioxidant combinatory therapeutic benefits greater than either glyburide or BA NPs alone. In another recent study, we converted the BA chemistry to BAM and conjugated AMD3100 on the surface (A-BAM NPs) for targeted delivery of NA1 to acidic ischemic tissue [135]. Recall, that in the recently completed ESCAPE clinical trial, NA1 failed to demonstrate therapeutic benefits when tPA was co-administered [45]. We found that intravenous administration of NA1-loaded A-BAM NPs can not only effectively improve the treatment of stroke, but also enable NA1 therapy to be compatible with tPA infusion.

4.1.4. Inorganic nanoparticles

Inorganic NPs refer to those NPs derived from inorganic materials, such as metals, semiconductors, and carbon. Compared to organic NPs, most inorganic NPs have a higher degree of stability and better-controlled tunability in size and shape. Some of inorganic NPs possess unique biological, chemical, electrical, and magnetic properties, making them attractive for specific biological applications. Among various inorganic NPs, NPs consisting of ceria have been extensively explored for treatment of ischemic stroke. Mechanistically, cerium ion at the surface of NPs can shift between the reduced (Ce^{3+}) and the oxidized (Ce^{4+}) forms through binding with oxygen, allowing ceria NPs to have both superoxide dismutase-mimetic and catalase-mimetic activities. Ceria NPs can protect cells from two major ROS, superoxide anion and hydrogen peroxide [136]. Kim and colleagues showed that intravenous

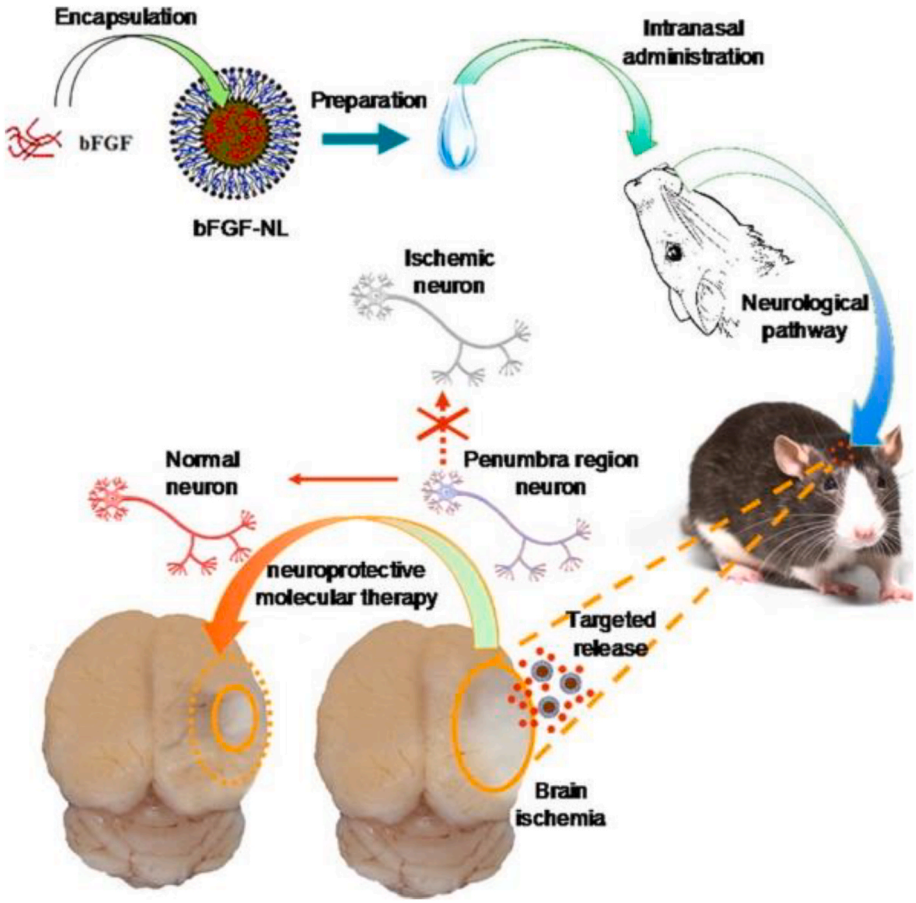


Fig. 5. Scheme of intranasal administration of bFGF-nanoliposomes (bFGF-NL) for targeted therapy of IR damage in a rodent stroke model resulting in reduced infarct volume. Reproduced from Ref. [130].

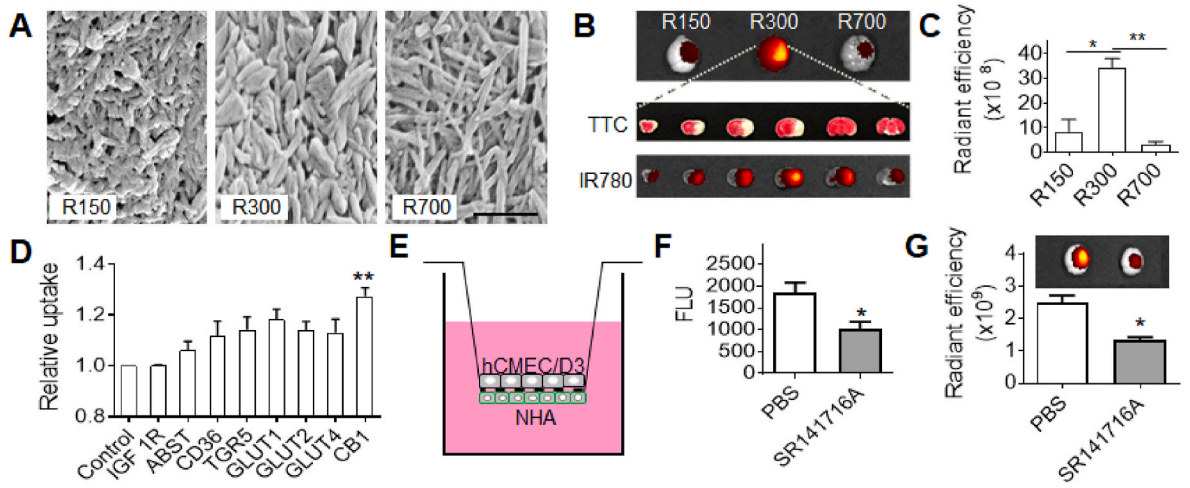


Fig. 6. BA NPs for drug delivery to the ischemic brain. (A) Representative SEM images of BA NPs. BA forms rod-shaped NPs in different sizes and shapes, designated as R150, R300, and R700, respectively. Scale bar: 500 nm. (B,C) Representative images (B) and semi-quantification (C) of BA NPs in the brains isolated from MCAO mice received the indicated treatment. (D) Flow cytometry analysis of the uptake of BA NPs in cells that were engineered to overexpress the indicated surface molecules. (E) Schematic diagram of *in vitro* BBB transcytosis assay. (F) *In vitro* analysis of the inhibitory effect of SR141716A on NP transcytosis. (G) Representative images (upper panel) and semi-quantification (bottom panel) of IR780-loaded BA NPs in the brains isolated from MCAO mice with and without pre-treatment of SR141716A. Reproduced from Ref. [133].

administration of PEGylated ceria NPs reduced infarct volume in a rat stroke model [137]. Bao and colleagues further engineered PEGylated ceria NPs through surface conjugation of Angiopep-2 and demonstrated that the resulting NPs penetrated the ischemic brain with a high efficiency. After intravenous administration, the NPs effectively improved stroke recovery and their efficacy was further improved through loading with edaravone [138]. To overcome the major challenges associated with ceria NPs for clinical translation, including short blood circulation time, aggregation, and uncontrollable catalytic reaction, the authors developed an strategy for *in situ* synthesis of bioactive zeolitic imidazolate framework-8–capped ceria nanoparticles (CeO2@ZIF-8 NPs) [139] (Fig. 7). They showed that the resulting nanosystem exhibited enhanced catalytic and antioxidative activities, increased the blood circulation time, and improved BBB penetration and accumulation in the brain. As a result, treatment with CeO2@ZIF-8 NPs notably decreased neuronal damage and suppressed inflammation and immune response.

Other than CeO_2 NPs, manganous tetroxide NPs (Mn_3O_4 NPs) have also been explored for stroke treatment. Compared to CeO_2 NPs, Mn_3O_4 NPs were shown to have greater antioxidase activities (SOD, catalase) [140,141]. Shi and colleagues developed an engineered nanosponge, Mn_3O_4@nanoerythrocyte-T7, and demonstrated that the nanosponge could remodel the ischemic microenvironment through self-adapted oxygen regulating and free radical scavenging, effectively improving post-stroke recovery [142]. More, recently, Wang et al. employed upconversion nanoparticles (UCNPs) to drive a nanophotosynthesis biosystem for local generation of oxygen and demonstrated this combination efficiently enhanced angiogenesis, reduced infarction, and facilitated brain tissue repair in a stroke mouse model [143].

4.1.5. Others

There are several other types of NPs, such as protein NPs,

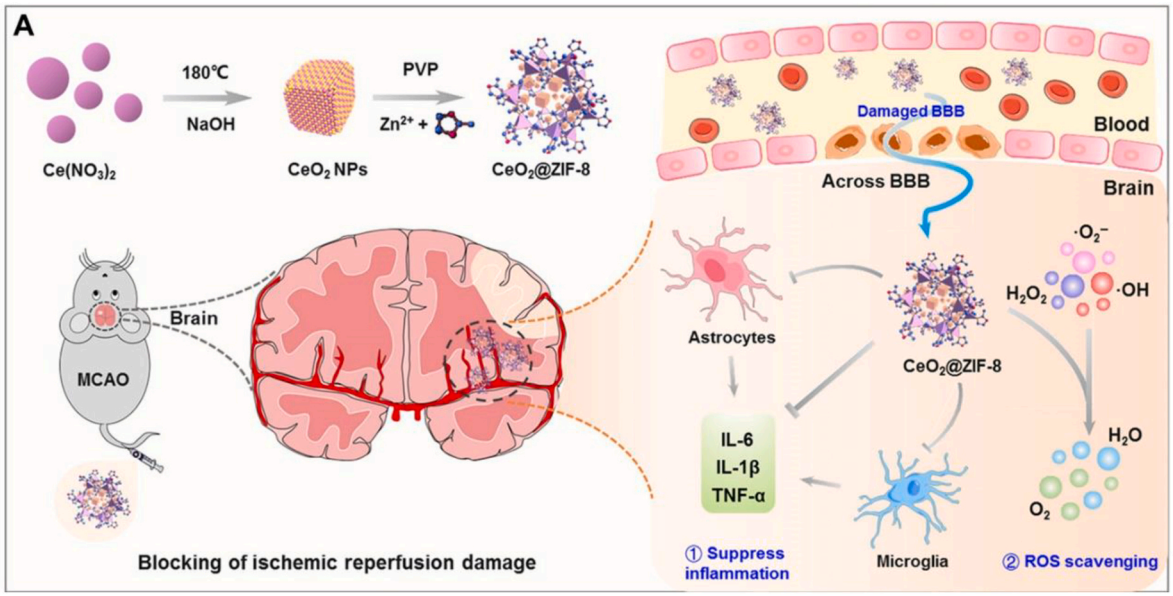


Fig. 7. Schematic illustration for *in situ* synthetic approach of CeO2@ZIF-8 nanotherapeutics and its neuroprotective application mechanisms against reperfusion-induced injury in AIS. Reproduced from Ref. [139].

nanoconjugates, and biomimetic NPs, which have been also evaluated for drug delivery to the ischemic brain for stroke treatment.

We recently designed and synthesized thrombin-activatable protein nanoparticles (APNPs) for the targeted delivery of NR2B9C, a neuroprotective peptide, to the ischemic brain (Fig. 8) [144]. The NPs were formed through the self-assembly of three recombinant polypeptides based on pairwise coiled-coil dimerization and further engineered for enhanced blood circulation and improved brain penetration through PEGylation and surface display of a transferrin receptor (TfR)-targeting peptide. Upon reaching the ischemic microenvironment, APNPs were cleaved by thrombin to release payload NR2B9C, which, in turn, effectively improve stroke recovery [144].

A variety of nanoconjugates have been developed and tested in murine stroke models. Adenosine is a nucleoside known to have potentially significant beneficial activity for many neurological disorders. Unfortunately, this compound is unsuitable for clinical translation because it is limited by its short plasma half-life and poor ability to penetrate the BBB. Gaudin and colleagues demonstrated that an adenosine bioconjugation with lipid squalene formed NPs, demonstrating prolonged circulation and neuroprotection in a mouse stroke model [145]. In another study, Zhang and colleagues reported an approach to selectively target inflammatory neutrophils using NPs composed of DOX-BSA conjugate [146]. They showed that treatment with these NPs inhibited neutrophil transmigration and decreased post-stroke brain damage [146].

More recently, various biomimetic vesicles have been developed for drug delivery to the ischemic brain. Among them, extracellular vesicles (EVs), which are cell-secreted nanoscale vesicles with subcellular structures, are particularly attractive as they often have great stability and long blood circulation time. Exosomes often carry microRNA and other cargo from the parent cells, making themselves alone a neurorestorative therapeutic. Applications of exosomes for the treatment of stroke have been reviewed in a recently published article [147]. However, clinical translation of exosomes has been limited by their low yield and purity as well as concerns about genetic materials within the vesicles. To overcome these limitations, other types of biomimetic vesicles, such as cell membrane-derived nanovesicles [148] and the fusion of cell membrane with liposomes [149], have been developed and shown great promise for drug delivery to stroke.

4.2. Engineering NPs for targeted drug delivery to the ischemic brain

Nanotechnology has the major advantage of manipulating the engineering of NPs for enhanced drug delivery to the ischemic brain. Various approaches, including surface functionalization, stimuli-responsiveness, and biomimicry, have been explored.

4.2.1. Surface functionalization for passive or active targeting

PEGylation through surface coating with polyethylene glycol (PEG) is a commonly used approach to passively improve the delivery of NPs to the ischemic brain [127–129,144,145]. In addition to reducing protein adsorption, PEGylation alters the composition of the protein corona to prevent non-specific cellular uptake [150]. As a result, PEGylation helps NPs to evade the mononuclear phagocyte system (MPS), resulting in prolonged blood circulation and increased potential to penetrate the compromised BBB.

However, passive targeting through PEGylation has limited efficiency, as the brain penetrability of PEGylated NPs solely depends on the

A

Name	Sequence
Coiled-coil motifs	
P1	NEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYK
P2	SIAATLENDLARLENENARLEKDIANLERDLAKLEREEAYF
P3	SPEDEIQQLEEEIAQLEQKNAALKEKNQALKYG
P4	SPEDKIAQLKQKIQALKQENQQLEEENAALEYG
P5	SPEDENAALEEKIAQLKQKNAALKEEIQALEYG
P6	SPEDKNAALKEEIQALEEENQALEEKIAQLKYG
Therapeutic peptides	
Tat- NR2B9c	YGRKKRRQRRRKLSSIESDV
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ
Responsive motifs	
Thrombin-	TPRSFL
MMP2-	PLGLAG
Others	
"self" peptide	GCCTAAAAGAGACCTACCTC
Flexible linker	SGPG
Target peptide	RTIGPSV

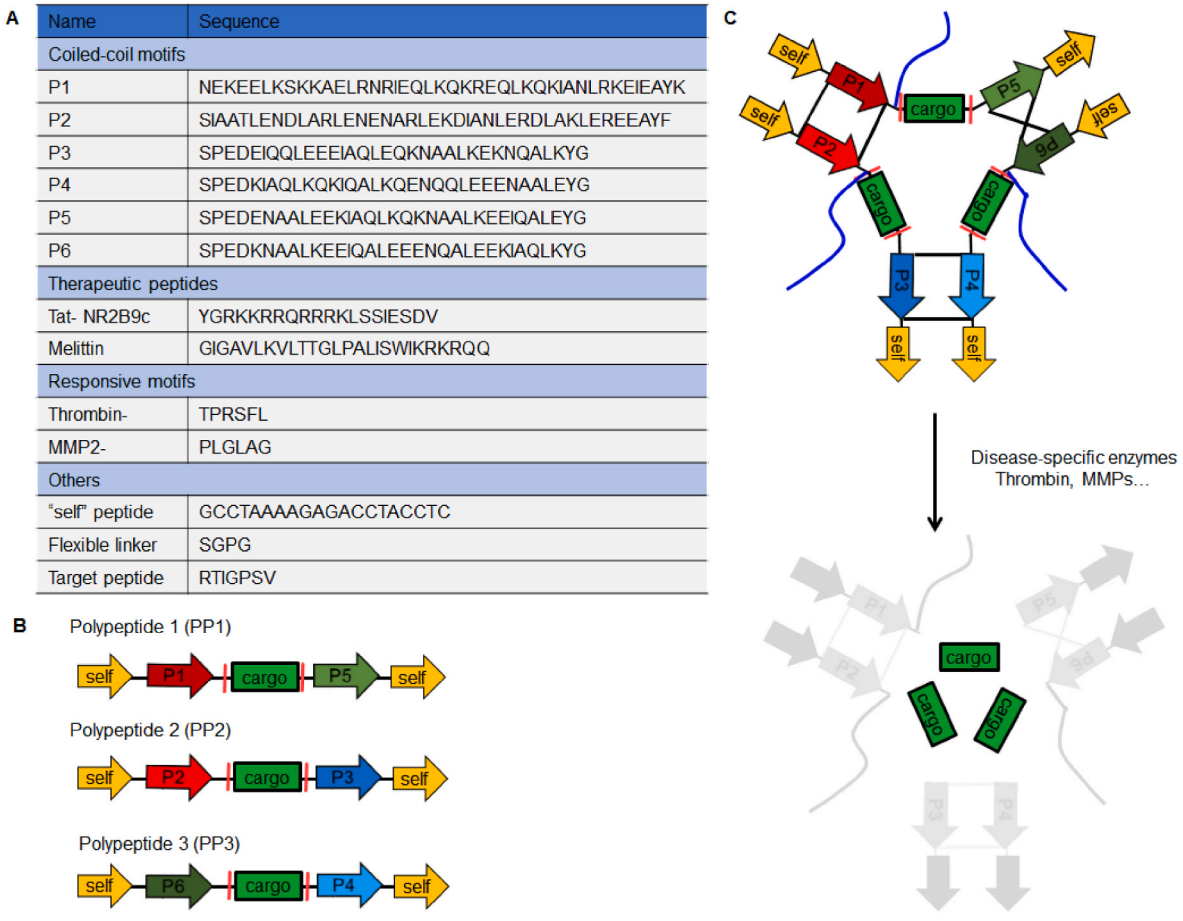


Fig. 8. Design of APNPs. (A) Function and sequences of modular peptides used in the study. (B) Schematic of polypeptides containing functional modular motifs. (C) Formation and activation of APNPs. Reproduced from Ref. [144].

degree of BBB disruption. Improving drug delivery to the ischemic brain could be potentially achieved through active targeting strategies via surface conjugation of ligands, which can interact with receptors or "receptor like" molecules highly expressed in the BBB, such as transferrin receptors (TfR) [151], or molecules enriched in the ischemic microenvironment, such as MMP-2 [106] and fibrin fibrils [152]. To identify molecular targets which are preferentially accumulated in the ischemic brain tissue, we recently profiled proteins in both the ischemic brain and normal brain using an antibody array, through which we identified 19 proteins that had significantly different levels of expression in the ischemic hemisphere, compared to those in the contralateral control. Further characterization identified CXCR4 as the most prominent target, which is highly expressed in the ischemic brain shortly after the onset of stroke and can be targeted by a well-characterized antagonist, AMD3100. We showed that surface conjugation of AMD3100 enhanced the accumulation of NPs in the brain by over 30-fold (Fig. 9a, b) [123]. In another study, we reported the development of multifunctional AMD3100-conjugated, shrinkable poly (2,2′-thiodiethylene 3, 3′-thiodipropionate) (PTT) NPs (ASPTT NPs) which are synthesized using a ROS-reactive PTT polymer and conjugated with AMD3100 [153]. This work showed that ASPTT NPs are capable of efficient encapsulation and delivery of glyburide to achieve anti-edema and antioxidant combination therapy, resulting in effective stroke treatment. Additional active targeting strategies were summarized in Table 2.

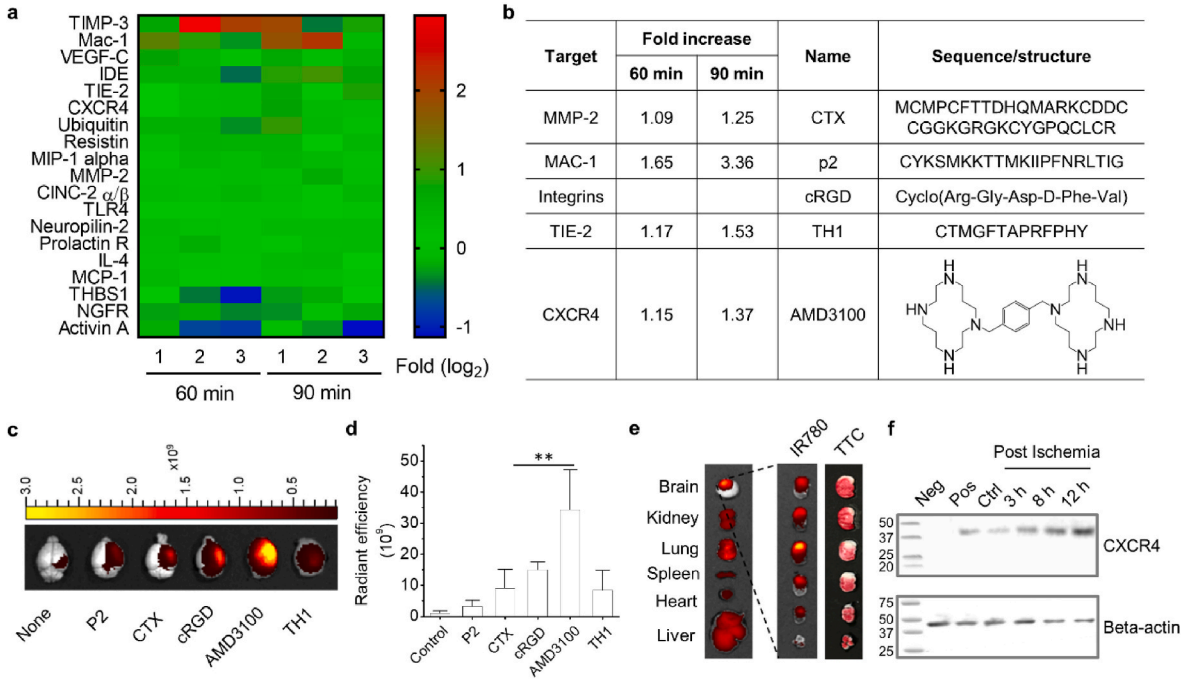
4.2.2. Stimuli-responsiveness

Despite great progress over many decades, accumulating evidence shows that active targeting is significantly complicated by the complex physiological system and limited efficiency for clinical translation [161]. Further improved targeted drug delivery requires engineering NPs to be "intelligent" and responsive to disease microenvironments by changing their physical or chemical structures [162,163]. To explore intelligent responsiveness as a novel approach to enhancing the delivery of NPs to the ischemic brain, we synthesized PCL-PEG based micellular NPs, which contain a thrombin-cleavable peptide that either expanded or shrank in size in response to thrombin cleavage (Fig. 10). We found that both the size expandable and shrinkable NPs penetrated the brain with greater efficiency than unresponsive NPs. Compared to the expandable nanoparticles, the size shrinkable nanoparticles were more efficient [123].

In addition to biological cues, intelligent NPs can also be designed to respond to physical or chemical stimuli in the ischemic brain. For instance, it is known that the blood vessels in the brain narrow due to thrombus formation leading to accelerated blood flow and higher fluid shear stress. This unique physical characteristic may provide an opportunity for targeted drug delivery. In a study by Korin and colleagues, a shear stress-targeting strategy was tested by synthesizing microscale aggregates of NPs, which break up into nanoscale components when exposed to abnormally high fluid shear stress. They found that after intravenous administration these shear-activated nanotherapeutics preferentially released payload tPA in the clotted area, inducing rapid clot dissolution and restoration of normal flow dynamics [164]. Similar thrombolytic approaches could be also employed for the treatment of ischemic stroke [165].

4.2.3. Biomimicry through surface coating with cell membranes

Biomimicry, which is often achieved through surface coating with cell membranes, allows for hijacking the biological interactions cells have with the host. This technique has recently emerged as a promising approach to engineering NPs for drug delivery to the brain. To date, various cell-based therapies have been explored for the treatment of ischemic stroke. Among them, neural stem cells (NSCs) are particularly attractive because they express various chemokine factors, such as CXCR4, and thus can efficiently migrate to the injured area [166]. To take advantage of the unique tropism of NSCs, we synthesized PLGA NPs and engineered them for targeted delivery to the ischemic brain through surface coating with the membrane of NSCs. We found that the membrane-coated NPs efficiently accumulated in the ischemic brain tissue after intravenous administration and the efficiency could be further improved using CXCR4-overexpressing membrane. We



Target	Fold increase		Name	Sequence/structure
	60 min	90 min		
MMP-2	1.09	1.25	CTX	MCMPCFTTDHQMARKCDDC CGGKGRGKCYGPQCLCR
MAC-1	1.65	3.36	p2	CYKSMKKTTMKIIPFNRLTIG
Integrins			cRGD	Cyclo(Arg-Gly-Asp-D-Phe-Val)
TIE-2	1.17	1.53	TH1	CTMGFTAPRFPHY
CXCR4	1.15	1.37	AMD3100	

Fig. 9. Identification and selection of ligands for targeted delivery of nanoparticles to stroke. (a) Heat diagram of proteins that are differentially enriched in the ischemic brain and in the control normal brain (*$P < 0.05$). (b) Candidate ligands that bind to the selected proteins. (c) Representative images and (d) semi-quantification of nanoparticles in the brains of MCAO mice received treatments with the indicated ligand-conjugated nanoparticles. (e) Representative images of brain slices with TTC staining (left) and fluorescence imaging (right). (f) Western Blot confirmed that the level of CXCR4 was significantly elevated in the ischemic brain. Negative control: 293T cell lysate. Positive control: U87 flank tumor lysate. Control: normal brain tissue. Reproduced from Ref. [123].

Table 2
Summary of ligands on nanoparticles for ischemic stroke.

Nanocarriers	Ligands	Targets	Cargo	Animal model	Application	Ref.
AMD3100-conjugated, size-shrinkable NPs (ASNPs)	AMD3100	CXCR4	Glibenclamide	Mice MCAO	High efficiency in penetrating the ischemic brain and low toxicity	[123]
Liposome (T7&SHp-P-LPs/ZL006)	T7 peptide and SHp	TfR and homed to ischemic brain tissue	ZL006	Rats MCAO	Penetrating the BBB and targeting the ischemic area	[154]
T7-conjugated PEGylated liposomes (T7-P-LPs)	HAIYPRH (T7) peptide	TfR	ZL006	Rats MCAO	Mediate the transport of nanocarriers across the BBB	[155]
Combining magnetic Fe3O4 NPs and RGD-modified dendrimers	RGD tripeptide	GPIIb/IIIa receptor expressed at the surface of the activated platelets	Nattokinase	Rats FeCl3-induced thrombosis	Targeted delivery to enhance the efficacy of site-specific thrombolytic treatment	[156]
Angiopep-2-PGP-STA-PEG-PAMAM NPs	Angiopep-2; PGP	Low density LRP1; CXCR2	STA	Rats, mice; MCAO	Dual-targeting delivery system; Improve their therapeutic effect against AIS	[157]
Anti-HSP72 vectorized liposomes	Anti-HSP72	HSP72	Citicoline	Rats MCAO	Targeted delivery to the peri-infarct region	[158]
tPA-loaded, cRGD-coated, PEGylated liposomes	Cyclic RGD	Activated platelets	tPA		Facilitate selective delivery and effective release of tPA at the site of thrombus	[159]
PLGA-CTX/LEX NPs	CTX	MMP-2	NEP1-40	Mice MCAO	The NPs efficiently and specifically accumulated in the brain's ischemic microenvironment	[106]
Fas ligand - conjugated PLNs loaded with NBP	Fas ligand antibody	Recruiting microglia	dl-NBP	Mice MCAO	The NPs specifically accumulated on microglia cells in ischemic region	[160]
Perfluorocarbon NPs	Anti-fibrin antibody and urokinase	Fibrin fibrils	Anti-fibrin antibody and urokinase	Dogs Femoral artery thrombosis	The fibrin-targeted urokinase NPs could evolve into an alternative to r-tPA for use in acute ischemic stroke victims	[152]

NPs, nanoparticles; MCAO, middle cerebral artery occlusion; TfR, Transferrin receptor; PEG, polyethylene glycol; RGD, arginine–glycine–aspartic; SHp, stroke homing peptide; LRP1, lipoprotein receptor-related protein 1; AIS, acute ischemic stroke; STA, scutellarin; PGP, N-acetylated proline-glycine-proline CTX, chlorotoxin; LEX, lexiscan; PLNs, PEGylated lipid nanoparticles; dl-NBP, 3-n-Butylphthalide.

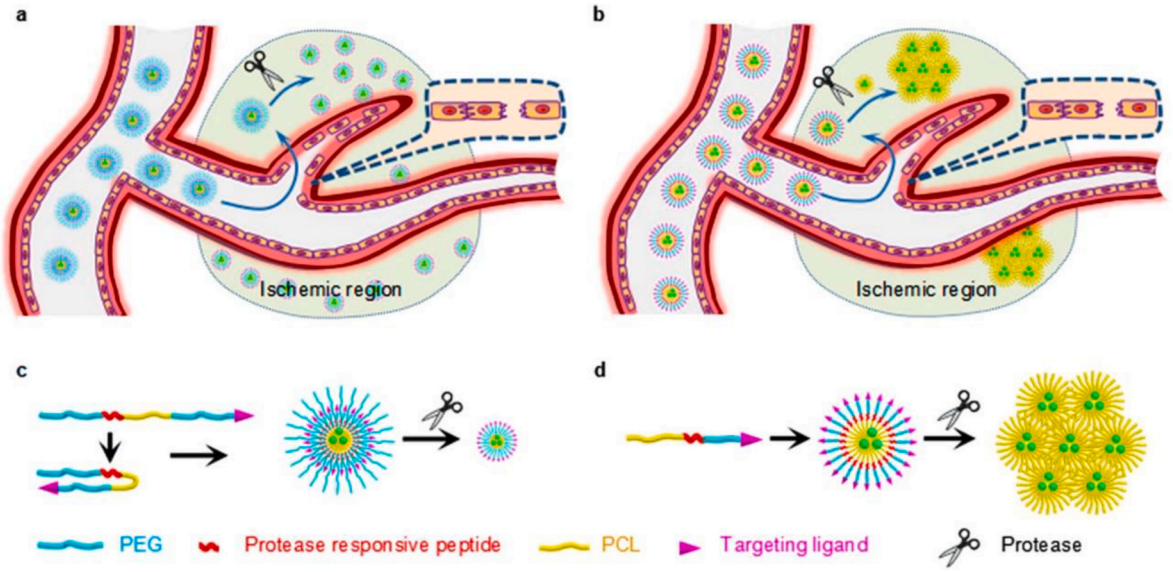


Fig. 10. Schematic diagrams of the intelligent responsiveness of (a, c) shrinkable and (b, d) expandable micellar nanoparticles in response to proteases enriched in the ischemic microenvironment. Reproduced from Ref. [123].

demonstrated that the resulting CXCR4-overexpressing NSC membrane-coated NPs enabled targeted delivery of glyburide to the brain for effective stroke treatment (Fig. 11) [167]. Similarly, Li and colleagues synthesized platelet membrane-coated magnetic nanoparticles and found that the resulting NPs inherited the natural properties of the platelet membrane and efficiently accumulated in ischemic stroke lesions after intravenous administration [168]. Lv and colleagues developed dextran NPs for drug delivery to the ischemic brain. These NPs possesses a surface coating of membrane isolated from red blood cell (RBC) which were shown to help prolong blood circulation life [169].

5. Perspectives and conclusion

Designing effective treatments through employing NP technology as drug delivery vehicles equally requires consideration of the physiochemical characteristics in additions to the payload. Improving targeted delivery to the ischemic brain could be potentially achieved by tuning these physical characteristics of NPs such as size, shape, and charge. NPs with a diameter smaller than 6 nm are subjected to rapid elimination by the kidneys while those larger than 50 nm are vulnerable to clearance by the reticuloendothelial system (RES) [170,171]. Compared to large-size NPs, NPs of smaller size have greater tissue penetration ability [172]. We recently showed that compared to non-responsive NPs, which maintained a diameter of ~120 nm,

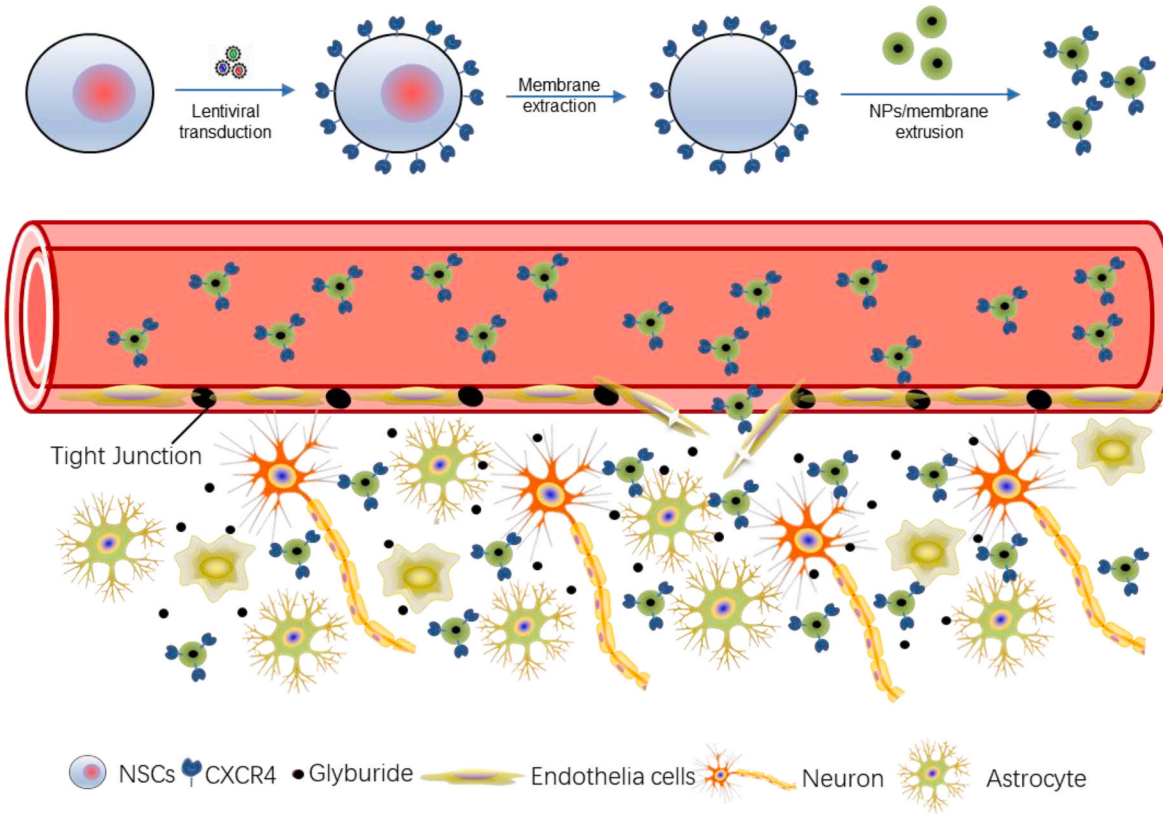


Fig. 11. Schematic diagram of targeted delivery of membrane-coated NPs to the ischemic brain through the surface coating of the membrane of CXCR4-overexpressing NSCs. NSCs are engineered through lentiviral transduction to overexpress CXCR4. Reproduced from Ref. [167].

thrombin-responsive NPs, which were ~200 nm in the circulatory system but shrank into 80 nm upon reaching thrombin-enriched ischemic brain tissue, demonstrated significantly greater stroke targeting efficiency [123]. The marked enhanced efficiency could be partially attributed to the greater ability of small NPs to penetrate the brain. Like size, the shape of the NPs also plays an important role in drug delivery. Compared to nanospheres, rod-shaped NPs were shown to have greater interactions with brain ECs and demonstrate a higher efficiency in brain penetration [173]. Among various sizes of rod-shaped NPs, we found that NPs of 315(l) x 60(d) have greater brain penetrability than both short (156(l) x 45(d)) or longer 730(l) x 35(d) NPs, suggesting that both the size and shape must be considered when designing optimal NPs for drug delivery to the brain (Fig. 7) [133]. In addition, the surface charge could also be a key parameter that determines the biodistribution of NPs. For example, Campos-Martorell and colleagues studied the impact of charge of liposomal NPs in an ischemic stroke rat model and found that neutral and negatively charged, but not positively charged NPs were able to penetrate the brain 90 min after intravenous administration [174].

Targeted delivery of NPs may also be achieved through external cues, such as magnetic force. It was reported that delivery of l-arginine to the ischemic brain was successfully improved through co-encapsulation with magnetic NPs and guidance by an external magnetic field. A similar approach could be employed to enhance the delivery of tPA for improved thrombolytic efficiency [175,176].

Currently, the standard care for ischemic stroke involves intravenous thrombolytic therapy and intravascular therapy, which, unfortunately, do not benefit most patients due to their narrow therapeutic windows. There are no FDA-approved pharmacotherapies that target improving post-stroke recovery. While further improved understanding of the biology of stroke is warranted, the lack of approved pharmacotherapies may not be solely due to the lack of therapeutic targets. In fact, numerous agents targeting a range of therapeutic targets have been tested in various clinical trials, including over 20 drugs tested in phase 2/3 clinical trials (Table 1). Alas, most of them failed to significantly improve clinical outcomes. The failure could potentially be attributed to the fact that most therapeutic agents cannot efficiently penetrate the brain. Owed to the great versatility in of NP engineering for brain targeting, adaptation to environmental stimuli, and BBB penetration, emerging nanotechnology has demonstrated great promise to overcome the drug delivery barrier (Table 2). However, the employment of NPs has major limitations in that it not only increases the complexity of therapeutic agents but also often leads to non-specific accumulation in organs associated with the reticuloendothelial system, mainly including the liver and the lung. As a result, the difficulty in GMP manufacture and quality control and concerns regarding off-target effects could prevent the clinical translation of NP-based stroke therapeutics. Development of the next generation of NPs with simple structures but potential for "intelligent" multifunctionality is greatly needed.

Ethics approval and consent to participate

Not applicable. This work is a review of existing published works.

CRediT authorship contribution statement

Bin Peng: Writing – review & editing, Writing – original draft, Conceptualization. **Farrah S. Mohammed:** Writing – review & editing, Writing – original draft, Conceptualization. **Xiangjun Tang:** Conceptualization. **Jia Liu:** Writing – original draft. **Kevin N. Sheth:** Writing – review & editing. **Jiangbing Zhou:** Writing – review & editing, Writing – original draft, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests.

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