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Advanced nano drug delivery systems for neuroprotection against ischemic stroke

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ABSTRACT

Ischemic stroke (IS) represents a significant threat to brain health due to its elevated mortality and disability rates. The efficacy of small-molecule neuroprotective agents has been impeded by challenges associated with traversing the blood-brain barrier (BBB) and limited bioavailability. Conversely, advanced nano drug delivery systems hold promise for overcoming these obstacles by facilitating efficient transportation across the BBB and maintaining optimal drug concentrations. This review aims to explore advanced neuroprotective nano drug delivery systems as a means of effectively administering neuroprotective agents to the brain using pharmaceutical approaches in the treatment of IS. By examining these systems, researchers and clinicians can gain valuable insights and innovative concepts, illuminating the potential of advanced neuroprotective nano drug delivery systems. Leveraging these advancements can drive the progress of pioneering and efficacious therapeutic interventions for IS.

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1. Introduction

Stroke, specifically ischemic stroke (IS), ranks as the second highest cause of adult mortality on a global scale. Annually, approximately 13.7 million individuals experience a stroke, resulting in 5.5 million deaths, with 87% of these cases attributed to IS [1,2]. IS is an acute brain disease caused by a sudden decrease in blood flow to the brain due to blood vessel narrowing or blockage [3]. Its extremely high rates of disability and death seriously endanger human health. Ischemia leads to irreversible and damaging infarct formation in the ischemic hemisphere and initiates a series of ischemic cascade responses, including energy depletion, ion imbalances and excitotoxicity, mitochondrial dysfunction, oxidative stress, cell death, and inflammatory responses [4–6]. In the realm of IS treatment, the early recanalization of occluded arteries through intravenous thrombolysis stands as the established clinical gold standard. Notably, the sole therapeutic approved by Food and Drug Administration (FDA) for IS treatment is recombinant tissue fibrinogen activator (rtPA) [7]. However, rtPA is often less effective because of its narrow therapeutic window (≤4.5 h), high patient-adaptation requirements, and short half-life [8]. Additionally, intravenous thrombolysis with rtPA increases the risk of hemorrhagic transformation and reperfusion injury [9]. Surgical thrombus removal is clinically indicated in very few patients, but the use of this treatment is limited [10].

Closure therapy is now globally recognized as the gold standard for the treatment of IS. However, it is crucial to acknowledge the occurrence of neuronal damage during IS pathogenesis and the irreversible neurological harm resulting from the embolization process, even after successful thrombolysis. This issue is often overlooked but of significant concern. The importance of neuroprotection during and following thrombolysis cannot be understated. In order to address these limitations, various neuroprotective agents, including oxygen carriers, excitatory inhibitors, antioxidants, anti-inflammatory agents, and stem cells, have been proposed for preclinical investigations. These agents hold potential for mitigating the damage caused by IS and warrant further explo-

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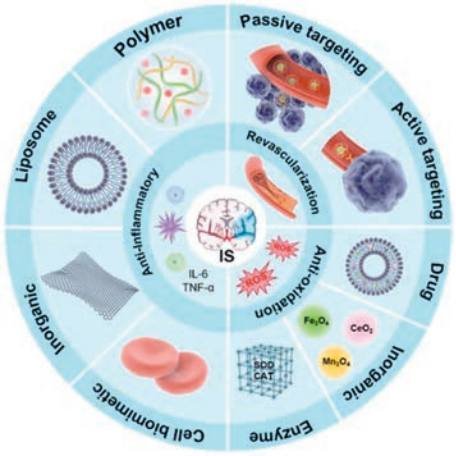

Scheme 1. Schematic diagram of an emerging approach for effective treatment of IS based on advanced neuroprotective strategies for nano-delivery systems.

ration in experimental studies [11]. Neuroprotective drugs, such as butalbital and edaravone (EDV) [12], have been recommended for use in clinical trials. Neuroprotective agents act on the pathophysiological processes underlying IS and reduce the functional damage to nerve cells [11]. Although the development of neuroprotective agents seems to have a positive role in the treatment of IS, most of these drugs have non-negligible drawbacks, such as a short half-life *in vivo* and the inability to cross the blood-brain barrier (BBB) to reach the site of the lesion, among other critical factors, which further impede the treatment of IS [13]. Hence, there is a pressing clinical demand for the development of novel and effective pharmacological neuroprotective therapies that can effectively slow down cerebral infarction, safeguard the penumbra (the region surrounding the core ischemic area), and minimize the neurological consequences of stroke. These therapies aim to provide enhanced protection to the brain tissue at risk, preserve neuronal function, and ultimately improve patient outcomes [14].

Neuroprotection-based advanced drug delivery systems (ADDSs) have attracted extensive interest in preclinical studies, including both targeted and responsive nanocarriers, which offer a distinct benefit in terms of precise drug delivery and controlled release [15]. In order to increase the effective concentration of neuroprotective agents in the brain, improve their brain-targeted delivery and control drug release, neuroprotective nanoparticles (NPs) have been designed as promising delivery systems, and these are expected to improve the therapeutic efficacy of IS. In this comprehensive review, we delve into the realm of advanced neuroprotective drug delivery systems to overcome the limitations in delivering neuroprotective agents to the brain for IS treatment [16]. We explore the application of pharmaceutical approaches to enhance the delivery of neuroprotective agents to the brain, emphasizing the role of advanced drug delivery systems. By understanding and harnessing the capabilities of these innovative drug delivery systems, new therapeutic strategies for IS can be developed. These systems offer the potential to optimize drug delivery, improve bioavailability, and enhance the efficacy of neuroprotective agents in treating IS. This review focuses on ADDSs based on neuroprotective agents for IS treatment. In addition, we discuss the potential and challenges of neuroprotective agent-based ADDSs applied in clinical studies, with the aim of providing new ideas and perspectives for IS neuroprotective treatment (Scheme 1).

2. Pathophysiology of IS

IS involves a complex set of pathophysiological mechanisms that interact and cause further brain damage. Understanding these can facilitate the search for and development of more ADDSs for neuroprotection against IS. Here, we explore the roles of neuronal damage, oxidative stress, inflammatory responses, and BBB integrity in IS pathogenesis (Fig. 1).

Within minutes of IS onset, glutamate over-release, accompanied by impaired re-uptake, results in the accumulation of large amounts of glutamate in the ischemic region due to hypoxic depolarization [17]. The excessive accumulation of glutamate in the extracellular space can further activate glutamate receptors (*N*-methyl-D-aspartate receptor (NMDA) and α-amino-3–hydroxy-5-methyl-4-isoxazole-propionicacid receptor (AMPA)) and dramatically disrupt a series of channels and glutamate transporter proteins, leading to excessive calcium, sodium, and water influx into neuronal tissues [18,19]. Elevated intracellular cytoplasmic calcium levels trigger a cascade of neurotoxic responses that initiate a series of events leading to cellular damage. These responses include decoupling of mitochondrial electron transport from adenosine triphosphate (ATP) production and decoupling of mitochondrial electron transport from ATP synthesis, as well as activation and overstimulation of a range of proteases and kinases. Consequently, these processes disrupt both the mitochondria and the nucleus, resulting in additional neuronal impairment and damage [19].

Oxidative stress is a key component in the pathogenesis of IS [20]. Following ischemia-reperfusion, oxidative stress is heightened due to mitochondrial dysfunction, surpassing the capacity of brain tissue to counteract reactive oxygen species (ROS) [21,22]. This triggers the activation of ROS-producing enzymes while reducing the activity on antioxidant enzymes. As a result, the delicate balance between the generation and scavenging of ROS and reactive nitrogen species (RNS) is disturbed, leading to their substantial accumulation.

ROS are generated through various mechanisms, including lipid peroxidation, protein denaturation, DNA modification, and the initiation of harmful redox-sensitive cell signaling pathways. These processes culminate in cellular apoptosis or necrosis, leading to detrimental outcomes [23,24]. Moreover, ROS and subsequent oxidative stress impair functional BBB integrity by disrupting its molecular organization and altering the expression of key tight junction (TJ) proteins [25]. Oxidative stress also decreases nitric oxide (NO) bioavailability in endothelial cells [26], and reduced NO availability exacerbates clotting, intensifies ischemic injury, and contributes to a further reduction in intracerebral blood flow. Furthermore, cells in an ischemic state can have elevated levels of calcium, sodium, and adenosine diphosphate, which increase mitochondrial oxygen production [27]. Reperfusion is a frequently employed treatment approach for IS, but it can also lead to the generation of ROS. As a result, oxidative stress is induced, immune cells are activated and aggregated, and the release of inflammatory factors is stimulated, further exacerbating the inflammatory response and ultimately leading to secondary damage [28].

Inflammation has a critical role in the progression of IS. Cerebral ischemic injury and subsequent reperfusion of blood flow induces the secretion of inflammatory cytokines and chemokines. Innate immune cells and microglia, in particular, have essential functions in post-ischemic inflammation, contributing to the inflammatory response associated with IS [29].

Following the occurrence of IS, a considerable number of neuronal cells that have undergone cell death release damage associated molecular patterns (DAMPs). These DAMPs play a role in activating innate immune cells during the acute and subacute phases of the condition [30]. Upon activation, receptors associated with these immune cells initiate downstream signaling pathways, including nuclear transcription factor-κB (NF-κB), mitogen-activated protein kinase, and type 1 interferon pathways. Subsequently, these activated pathways trigger an upregulation of proin-

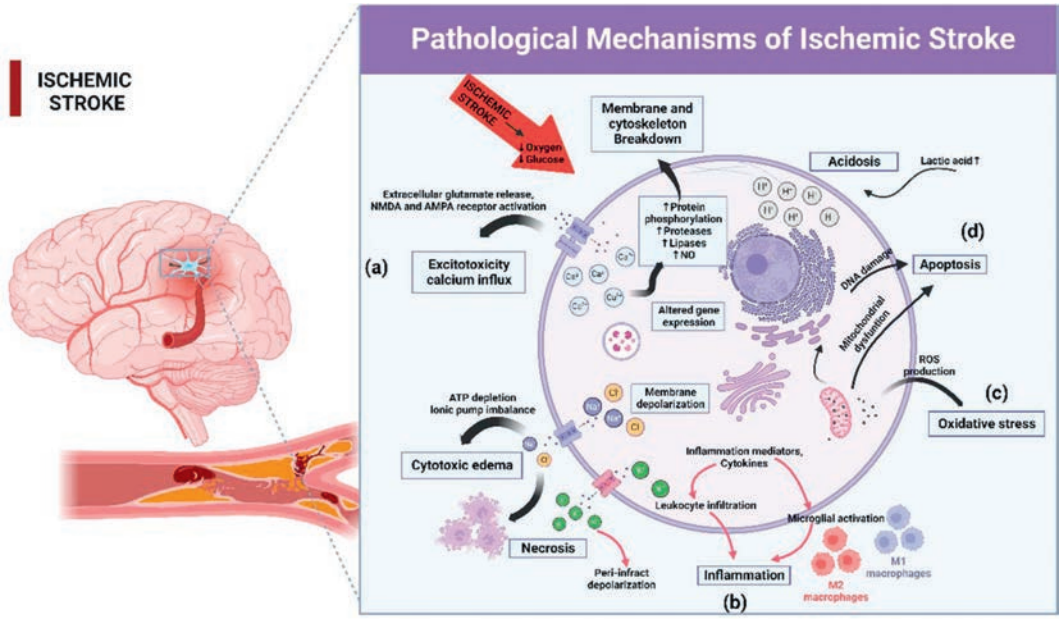

Fig. 1. Schematic diagram of the pathological mechanisms following the onset of IS and the cascade of injuries. First, (a) calcium overload triggers neurotoxicity, followed by cytotoxic edema, which then triggers (b) inflammation, which produces (c) oxidative stress damage and then (d) apoptosis.

flammatory cytokines, chemokines, and oxidative metabolites. This exacerbates neuronal damage by promoting inflammation and oxidative stress in the affected area [31].

During ischemic injury, microglia are rapidly activated and undergo morphological changes that promote their proliferation and production of inflammatory factors [32]. There are two distinct subtypes of activated microglia: the proinflammatory M1 subtype and the anti-inflammatory M2 subtype [33]. M1 microglia are characterized by their ability to produce a range of proinflammatory cytokines including tumor necrosis factor as well as interleukins. On the other hand, M2 microglia are identified by their secretion of anti-inflammatory or neuroprotective factors, including interleukin-10 (IL-10), transforming growth factor-β (TGF-β), insulin-like growth factor 1 (IGF1), and arginase-1 (Arg1) [34].

These factors can inhibit brain injury and promote tissue recovery, and ischemia and reperfusion can activate perivascular macrophages. These activated macrophages release proinflammatory cytokines, promote endothelial adhesion molecule expression, facilitate increased leukocyte infiltration (particularly neutrophils), and contribute to BBB injury [35]. The substantial influx of neutrophils within a short timeframe results in protease release, which further disrupts BBB integrity and exacerbates oxidative stress, leading to severe brain injury [36].

An essential role is played by the BBB in maintaining the balance of the neuronal microenvironment in the central nervous system and in avoiding the invasion of foreign substances. It accomplishes this by regulating the permeability of substances through TJ protein complexes [37]. Together, they limit the diffusion of substances through the paracellular pathway between endothelial cells in brain capillaries. This selective control over the transport of molecules ensures the integrity and proper functioning of the BBB in maintaining the brain's internal environment [38].

During IS, the BBB sustains damage, particularly during the early phase of reperfusion (within 6 h). This increased BBB permeability, during this phase, is primarily influenced by endothelial cell transport mechanisms, possibly involving vesicle transport [39]. In the later stages, the substantial release of chemokines, cytokines, and other pro-inflammatory mediators further activates microglia and increases BBB permeability. Disruption of TJ integrity leads to infiltration of a variety of immune cells. These immune cells release a variety of neurotoxic and neurotrophic factors that can have diverse effects on the nerves-protective or destructive [40–43].

As previously discussed, IS disrupts BBB and increases vascular permeability. This provides an opportunity to design nanomedicine carriers that leverage the physiological changes in the BBB to facilitate drug delivery to the ischemic site for protective effects. Currently, there are four main transportation routes to consider: receptor-mediated metabolism (RME), adsorption-mediated metabolism (AMT), intercellular lipophilic transport, and nasal administration bypassing the BBB. (1) RME: This mechanism relies on ligand-modified nanocarriers that bind to specific receptors expressed in the brain, facilitating their transport across the BBB with high targeting precision. Various receptors, such as transferrin (Tf), low-density lipoprotein (LDL), and nicotinic acetylcholine (nACh) receptors, are expressed on brain capillary endothelium [44]. Utilizing ligand-modified nanocarriers to exploit receptor-mediated transcytosis proves effective in enhancing brain targeting efficiency. Notably, stroke-homing peptide (SHp), a commonly used targeting peptide, has been shown to significantly enhance endocytosis mediated by transferrin receptors. For instance, a recent study by Yang *et al.* employed SHp peptide-functionalized cyclodextrin-derived ROS-responsive nanocarriers loaded with DL-3-*n*-butylphthalic acid (NBP). This modification enabled wider distribution, enhanced NBP delivery, improved neuronal recovery at the injury site, and promoted functional recovery [45]. (2) AMT: In AMT, cationic polymers and cell-penetrating peptides (CPPs) interact with negatively charged endothelial cells' surfaces through nonspecific electrostatic interactions, enabling penetration of the BBB [46]. Additionally, positively charged molecules like cationic bovine serum albumin (BSA) can be used to modify nanocarriers. For example, Hou *et al.* designed a MnO_2 nano-delivery system modified by BSA, demonstrating not only excellent biocompatibility but also effective BBB penetration. This modification allowed for non-invasive imaging of BBB permeability in MCAO rats, offering brain targeting efficiency and high selectivity [47]. (3) Intercellular lipophilic transport: This approach involves nanocarriers designed to mimic cell membrane properties to aid drug passage through the BBB. Lipid-soluble small molecules with a molecular weight less than 500 kDa can passively

diffuse across the BBB [48]. Liposomes, known for their biocompatibility and high lipophilicity, are suitable for drug delivery due to their low cost and easy preparation. However, to enhance their ability to transport drugs to the brain, liposomal carriers can be modified with ligands and cationic polymers to improve targeting [49]. (4) Nasal administration bypassing BBB: Recent interest has grown in delivering drugs to the brain *via* nasal administration. For example, Zhang *et al.* [50] designed a SPNP thermosensitive hydrogel with dual-targeting. This nanoparticle was modified with SS-31 targeting peptide for mitochondria and exhibited robust ROS-responsiveness while encapsulating puerarin (PU). By incorporating SPNP into a hydrogel for nasal administration, it effectively circumvented the BBB. This approach enhanced drug targeting to the ischemic site in MCAO rats, offering multiple benefits such as ROS-responsiveness, mitochondrial targeting, and antioxidant effects. Notably, the hydrogel improved drug bioavailability and reduced loss compared to conventional formulations.

3. Advanced neuroprotective drug delivery systems for IS

3.1. Advanced anti-inflammatory drug delivery systems

Inflammation plays an important role in IS pathological processes and treatment [51]. First, cerebral ischemia leads to mitochondrial dysfunction, triggering a series of cascade responses and the massive accumulation of inflammatory cytokines in the ischemic core. In contrast, during ischemia–reperfusion, peripheral immune cells are promptly activated and infiltrate the ischemic core and the surrounding semi-dark zone driven by an inflammatory gradient, thereby contributing to secondary damage. Cytokines, adhesion molecules, and NO play crucial roles in mediating inflammatory injury, as they are produced soon after the onset of ischemia and can induce irreversible damage [22]. Both inflammatory and immune responses markedly amplify brain injury; therefore, it is important to design effective nanocarriers loaded with anti-inflammatory drugs to treat ischemic brain injury (Table S1 in Supporting information).

3.1.1. Polymer-based nanocarriers

Polymer-based nanocarriers offer promising prospects for addressing the treatment of inflammation in IS and facilitating the delivery of neuroprotective drugs. These nanocarriers exhibit favorable attributes, including good biocompatibility and the presence of abundant functional groups. In recent years, polymeric nanoparticles have gained significant attention as delivery systems for IS treatment. Among these, poly(lactic-*co*-glycolic acid) (PLGA) has emerged as a widely used material in stroke therapy. This is primarily due to its desirable properties, such as non-toxicity, biocompatibility, and biodegradability. The utilization of PLGA-based nanocarriers holds great potential for enhancing therapeutic outcomes in IS by enabling efficient drug delivery while ensuring compatibility with biological systems [52].

Dang *et al.* [53] developed a dual-targeting drug nanoformulation using terminal hydroxy polyamidoamine dendrimers (PEG-G5.0 PAMAM NPs) loaded with calendula to target sites of ischemic brain inflammation for anti-inflammatory and anti-apoptotic purposes. This nano-formulation was further modified with two targeting peptides, the low-density lipoprotein receptor (Angiopep-2) and neutrophils. Leveraging the high BBB-transport capacity of Angiopep-2 and the affinity of *N*-acetylated PGP for CXCR2 receptors expressed on infiltrating neutrophils, the nanoformulation exhibited enhanced efficiency in crossing the BBB and demonstrated superior anti-stroke activity [54]. Multiple molecular mechanisms mediate the therapeutic effects of this agent. These involve the downregulation of inflammatory cytokine expression, inhibition of neutrophil infiltration, reduction of intracellular calcium overload, and blockade of the inflammatory HMGB1/TLRs/MyD88/TRIF/NF-κB signaling pathway. Importantly, *in vitro* models demonstrated that the polyethylene glycolylated PAMAM dendrimers employed in the nano-formulation do not compromise BBB integrity. Furthermore, the nano-formulation exhibited no cytotoxicity in oxygen glucose deprivation (OGD) model, suggesting their potential for treating IS safely and effectively.

Lu *et al.* [55] designed a PARA-PEGylated NP, incorporating an ROS-scavenging polymer (C-PEG-LysB) and a fibronectin-binding peptide (CREKA, referred to as peptide C). Through rapamycin loading, the microglia phenotype was polarized from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, downregulating the level of inflammation Under ischemic conditions, this combination, resulting in ROS elimination and the disruption of stress signaling pathways, was found to enhance neuronal survival. Further, micelles exhibited a prolonged circulation time and lower blood clearance rates than free rapamycin.

Polymeric NPs also provide ideas for nanotherapeutics for a variety of neurological disorders. These biodegradable polymeric NPs are nontoxic, biocompatible, and exhibit sustained-release properties, thereby enhancing the therapeutic effectiveness of anti-inflammatory drugs within the body [56]. The size of polymer micelles less than 100 nm makes them circulate stably in the blood for a long time, for different polymer nanoparticles, they can help the drug targeting to the target cells by attaching different targeting parts, such as common folic acid and transferrin, so as to enhance the cellular uptake and the accumulation of drugs in the ischemic site. Its special shell-nucleus structure, which can wrap the different properties of the drug, is widely used for delivering hydrophobic. Its special shell-nucleus structure can encapsulate drugs of different properties, which is widely used for delivering hydrophobic drugs, metal complexes, *etc*. It is easy to carry out functionalization modification to improve the bioavailability of drugs and reduce immunogenicity. However, they also have certain limitations, such as high manufacturing costs, the need for specialized skills during the manufacturing process, and the potential toxicity associated with the organic solvents used in the formulation. Currently, one of the main problems faced by polymeric micelle delivery systems is the scalability of their production. In the laboratory, micelles with high drug loading and good stability can be prepared, but in large-scale production, due to the limitations of environmental factors and other constraints, it may not be possible to produce micelles with the same quality and specifications, which cannot fully satisfy the requirements of clinical drug delivery. Based on this, there is a need for an in-depth study of the different properties of polymer micelles to control the chemical-physical properties of polymer micelles for effective delivery of anti-IS drugs.

3.1.2. Lipid-based nanocarriers

The creation of liposomes has transgenerational implications for drug delivery nanocarriers [57]. These nanovesicles comprise concentric lipid bilayers [58] and offer several advantages for drug encapsulation and delivery. They can carry lipophilic substances, such as lipophilic drugs and proteins, within their lipid membranes, as well as hydrophilic molecules within the aqueous compartment [59]. Consequently, lipid-based delivery systems demonstrate remarkable drug-carrying capacity and high biocompatibility, particularly as carriers of poorly water-soluble drugs [60]. Moreover, liposomes are biodegradable and exhibit exceptional biocompatibility, and they can efficiently traverse the BBB, making them particularly attractive to researchers for the treatment of inflammatory damage associated with IS. The unique properties of liposomes have attracted significant attention in the pharmaceutical industry as a promising approach to drug delivery and therapeutic applications in the context of IS [61,62].

Significant advancements have been made in lipid-based therapeutic approaches for IS. Wang *et al.* [63] investigated various liposome formulations. They developed polyethylene glycol (PEG)/cRGD double-modified liposomes encapsulating 9-AA (9-AA/L-PEG-cRGD) to enhance the *in vivo* circulation time of 9-AA, improve its biodistribution, and increase its accumulation in the brain. 9-AA upregulates NR4A1 expression and can attenuate the production of proinflammatory mediators. It achieves this by downregulating the expression of pro-inflammatory markers, such as tumor necrosis factor-α (TNF-α), IL-1β, and cyclooxygenase-2 (COX-2), in microglia. This modulatory effect on inflammatory responses is neuroprotective, specifically by countering inflammation-induced apoptosis. The liposome delivery system developed by Wang *et al.* demonstrates both anti-neuroinflammatory and anti-ischemic brain injury effects. In mice subjected to MCAO and treated with 9-AA/L-PEG-cRGD double-modified liposomes *via* intraperitoneal injection, a dose-dependent reduction in the infarct volume was observed. The liposomes also exhibited superior neuroprotective effects compared to those with the administration of free 9-AA alone. This research highlights the potential of the 9-AA/L-PEG-cRGD liposome formulation as a promising strategy for mitigating neuroinflammation and reducing brain injury in the context of IS [64].

The use of liposomes as nanocarriers can help achieve effective drug encapsulation rates, good particle size dispersion, and long-term drug stability. Regarding drug delivery, liposomes have been widely found to improve pharmacokinetics and biodistribution. Despite the advantages of liposomes, no liposome-based nanocarriers are currently available for clinical IS treatment. At the same time, the preparation of liposomes requires complex production methods that may not be conducive to their large-scale production. When directly compared to that with conventional IS drugs, none of the liposomal therapeutics currently on the market result in an overall survival benefit, suggesting that this could be due to lipid instability, drug leakage, or inadequate targeting. Therefore, we need to develop better techniques to enhance the stability of liposomes for better drug targeting as well as drug release.

3.1.3. Inorganic nanocarriers

Inorganic nanomaterials, such as silica and iron oxide, have demonstrated significant potential in the field of IS treatment [59]. Kang *et al.* [65] investigated the therapeutic effects of magnetosome-like ferromagnetic iron oxide nanocubes (FIONs) loaded into human embryonic stem cells to generate spherical neural masses (SNMs). Targeted SNM delivery was found to attenuate the inflammatory cascade response associated with secondary injury in intracerebral hemorrhage and improve the early neurological status.

In another study, Cha *et al.* [66] developed a lipid-coated magnetic mesoporous silica NPs (LMCs) based on cerium dioxide nanoparticles (Ce NPs) with ROS scavenging ability and enhanced magnetic resonance imaging (MRI) efficacy. *In vivo*, the intracerebroventricular injection of LMCs resulted in their accumulation in the perihematoma region, where they were phagocytosed by macrophages, leading to a reduction in inflammatory macrophage infiltration and a subsequent decrease in the infarct size. These studies highlight the potential of inorganic nanomaterials as therapeutic agents for IS treatment. The utilization of magnetosome-like FIONs and lipid-coated magnetic mesoporous silica NPs doped with Ce NPs shows promise in modulating inflammatory responses and reducing brain damage associated with IS [67].

Inorganic nanocarriers offer advantages over polymeric NPs in terms of size and shape control, as well as their simple preparation and functionalization processes. Notably, inorganic nanocarriers can be traced easily using microscopic methods, including transmission electron microscopy, MRI, and analytical methods [68]. However, inorganic nanocarriers also have disadvantages, such as their inherent toxicity and low biocompatibility, which require further studies.

3.1.4. Cell-derived biomimetic nanocarriers

Unlike NPs synthesized *in vitro*, bionanocarriers offer improved biocompatibility and reduced toxicity [69], making them attractive options for drug delivery. One approach gaining attention is camouflaging nanocarriers using natural cells or cell membranes. This strategy bypasses recognition by the mononuclear phagocyte system, resulting in reduced immunogenicity and an extended circulation time [70]. Drawing inspiration from the affinity of neutrophils for inflammatory cytokines, Liu [71] proposed a "nano-buffering" strategy mimicking neutrophils. This approach involves attaching nanocarriers to the core of damaged neurovascular units through receptor-mediated inflammatory pathways and integrin-mediated adhesion. This attachment helps to alleviate destructive erosion from the core to surrounding areas. Cannabidiol (CBD), a promising natural product with neuroprotective properties, was encapsulated in PLGA NPs as the inner core. Subsequently, the NPs underwent a coating process involving a neutrophil membrane (NM) in order to enhance their targeting capabilities towards the ischemic site. To harness the ROS-scavenging effects of alpha-lipoic acid (LA), it was surface-modified onto NM-NP/CBD through hydrophobic modification, resulting in the formation of the fundamental unit known as LA-NM-NP/CBD NPs. Upon *in vivo* administration, LA-NM-NP/CBD exhibited properties akin to a nanobuffer within the ischemic brain. Moreover, membrane cross-fusion induced by dynamic ring-opening polymerization could further enhance the role of nanobuffer layers between neighboring units *in vivo*. Intravenous injection of NPs in mice resulted in neuroprotective effects and ROS-scavenging activity, highlighting its potential for IS treatment (Fig. 2).

Although many anti-inflammatory drugs have shown neuroprotective effects in preclinical studies and in IS treatment, the potential neurotoxicity of nanomedicines when they enter the brain through the BBB must be considered. NPs can interact with neuronal cells and microglia, potentially causing neurotoxicity [74]. The utilization of various nanomaterials in the treatment of IS has shown promising efficacy during the preclinical phase. However, it is crucial to consider several factors beyond these initial positive outcomes. First and foremost, the potential toxicity associated with these nanomaterials themselves, as well as any synergistic toxicity that may arise when combined with drugs, must be thoroughly evaluated. Another critical aspect to address is the biodegradability of the nano-delivery system when navigating BBB for treatment. It is imperative to ensure that the nanomaterials can be metabolized by the organism after releasing the drugs into the brain, thus preventing any potential toxicity. In this regard, commonly used nanomaterials like polylactic acid and polyglycolic acid have been verified to possess excellent degradability. Particularly, PLGA has found extensive applications in drug delivery and tissue engineering due to its commendable biocompatibility. It is worth noting that during the pre-design phase of nano-delivery systems, various properties of nanomaterials should be carefully considered to ensure the safety of drug delivery. This includes an assessment of the physicochemical properties of nanomaterials, their potential toxicity, routes of exposure, and *in vivo* behavior. Additionally, controlling the dimensions of brain-targeted nano-delivery systems and the properties of modifiers and ligands used for surface modification can significantly influence the biosafety of nanomaterials. Finally, when validating these delivery systems for dosage in animal models, it becomes essential to explore strategies to minimize systemic toxicity while maintaining effective drug concentrations. Furthermore, the substances encapsulated by NPs, such as cell membranes, could have specific neurotoxic proper-

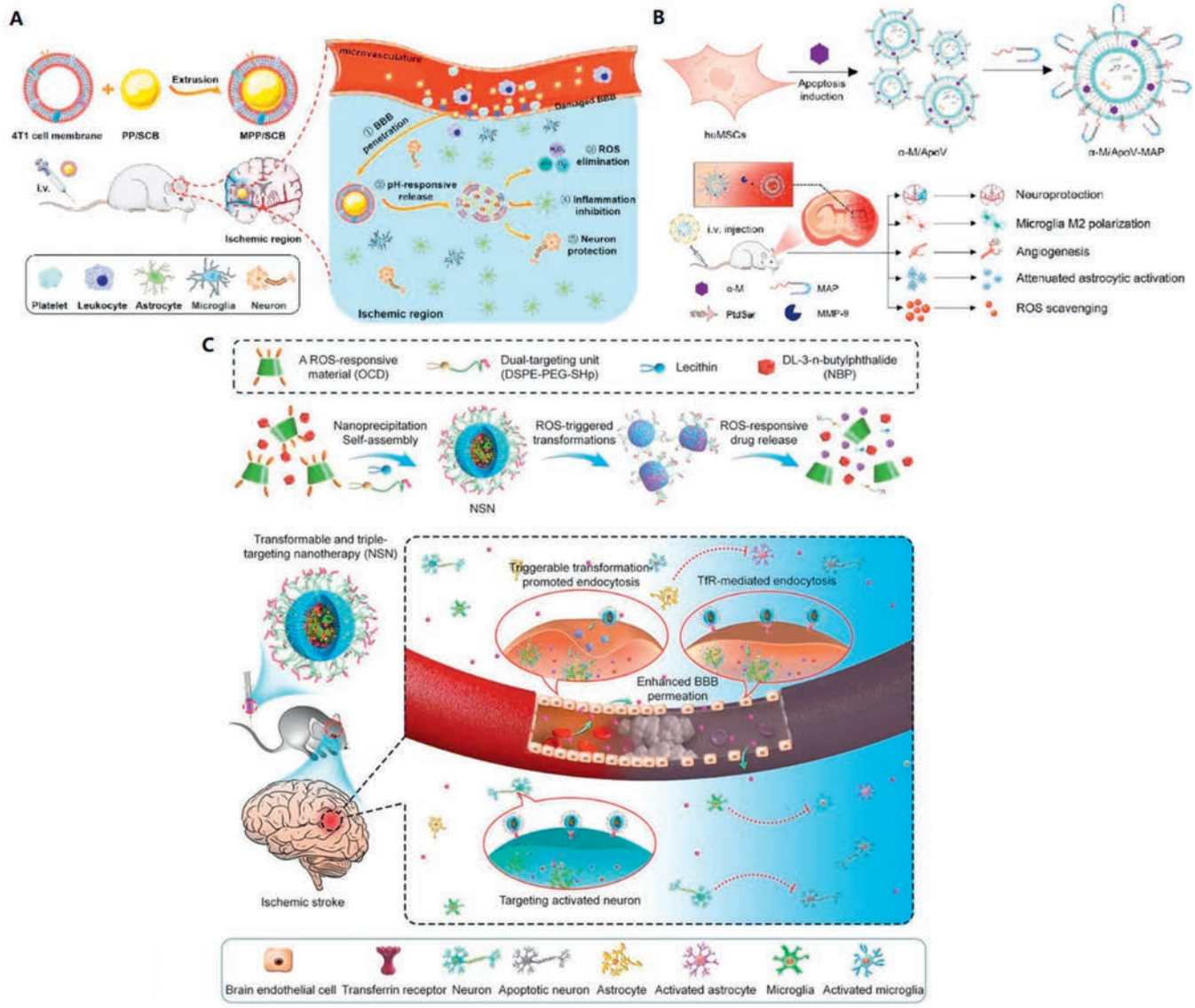

Fig. 2. Advanced anti-inflammatory drug delivery systems. (A) 4T1 cell membrane camouflaged pH-sensitive succinyl alcohol polymer nanoparticles that cross the BBB through the cell membrane camouflage and receive stroke pH responsive release for anti-inflammatory neuroprotective effects. Copied with permission [72]. Copyright 2021, American Chemical Society. (B) Schematic representation of stem cell-derived apoptotic vesicles for effective treatment of IS by loading α-M. Reproduced with permission [73]. Copyright 2023, American Chemical Society. (C) Schematic diagram of the treatment of IS targeting anti-inflammatory ROS-responsive, translatable and multi-targeted nanotherapeutic agents. *In vivo* targeted treatment of IS with the properties of ROS-responsive releasing drug butylphthalide, enhanced BBB permeability and neuronal targeting ability, exerting powerful anti-inflammatory ability. Reproduced with permission [45]. Copyright 2023, American Chemical Society.

ties. For example, platelets contain and secrete various substances that can cause neuronal dysfunction. When NPs are encapsulated using immune cell membranes, which interact with the immune system and promote an inflammatory response, they may move in an unfavorable direction. Additionally, there are modifications involving the use of cell membranes tailored to specific applications, such as the development of bionic nanocarriers for glioma treatment. These nanocarriers achieve precise brain targeting by utilizing specific ligands present on the membranes of microglial tumor cells. Another approach involves leveraging exosomes produced by cancer cells for drug delivery. While these methods have shown promising brain-targeting effects, it is imperative to conduct comprehensive research to ascertain whether these carriers have completely shed the characteristics of cancer cells, thus ensuring their biosafety. In addition, there are inorganic metallic materials that hold significant promise in therapy due to their inherent properties. Emerging nanomaterials like nano-enzymes and metal-organic frameworks (MOFs) are increasingly employed in IS therapy because of their capacity to mimic the enzymatic activities of superoxide dismutase (SOD) and catalase (CAT). However, it is essential to note that most inorganic metals, with the exception of silica, which has demonstrated both biocompatibility and degradability, have the potential to be biotoxic. Moreover, ensuring the stability of these inorganic nanomaterials in solution remains a major challenge. This stability is critical to achieving effective drug loading and controlled release at specific locations within the brain. Therefore, it is crucial to carefully evaluate the neurotoxicity of nanomedicines and understand their potential interactions with neuronal cells, microglia, and the immune system. Further research is required to ensure the safe and effective use of nanomedicines for the treatment of IS and other neurological conditions [75].

3.2. Advanced drug delivery for revascularization

IS arises from the occlusion or blockage of cerebral vessels, leading to cerebral infarction. The extent and neurological outcomes of cerebral infarction following focal cerebral ischemia are determined by the duration and severity of the ischemic event and the specific location of the infarct area [76,77]. After an IS, regions of the brain affected by ischemia undergo a series of changes and vascular remodeling processes (Table S2 in Supporting information).

Vascular regeneration plays a critical role in the treatment of ischemic diseases by facilitating the formation of new capillaries from pre-existing blood vessels [78]. Angiogenesis, the process of generating new blood vessels, is a highly regulated and multi-step phenomenon that is crucial for tissue growth, development, and repair [79–82]. In normal physiological conditions, angiogenesis is tightly controlled through a complex interaction of pro- and anti-angiogenic factors. Key factors involved in this process include a variety of growth factors that help maintain capillary stability [83]. These factors orchestrate the intricate balance between promoting the growth of new blood vessels and stabilizing the existing vasculature [84,85]. These factors, along with other pro-angiogenic cytokines, exist in a delicate balance within the circulation. However, disruption of this homeostatic relationship can occur, particularly in the presence of tissue hypoxia, which can stimulate angiogenesis and lead to increased proangiogenic factor production [86].

The inherent slowness of the spontaneous vascular regeneration response necessitates the exploration of strategies to expedite this process. Importantly, the formation of new blood vessels involves the concerted effects of multiple angiogenic factors, rather than a single factor. Achieving optimal angiogenesis requires the precise spatiotemporal coordination of pro-angiogenic factors [87,88]. NPs offer a promising avenue for controlling the release of growth factors, thereby acting as effective drug carriers that can simultaneously deliver multiple therapeutic agents for IS treatment. Furthermore, the rational design of nanomedicines employing both passive and active targeting strategies can address various ischemic cascade events, including oxidative stress, inflammation, cell death, and tissue repair. Nanomedicine can mitigate these detrimental processes and facilitate tissue recovery by specifically targeting ischemic tissues.

3.2.1. Passive targeting drug delivery system

During IS, the ischemic condition causes disruptions in vascular function, leading to increased vascular permeability. In recent years, NPs have emerged as promising tools for improving drug delivery to ischemic tissues. This is attributed to their unique physicochemical properties and the ability to control particle sizes. The advantageous characteristics of NPs, such as their small size, high surface area-to-volume ratio, and potential for surface modifications, allow for efficient drug encapsulation and targeted delivery to the site of ischemia. These properties enhance the therapeutic efficacy of drugs and minimize off-target effects, making NPs a promising approach for improving drug delivery in the context of IS [89]. For IS treatment, the NP-based delivery of angiogenic genes has been developed to circumvent the risk of gene degradation. Previous research has highlighted the crucial role of endothelial cells [90] and endothelial progenitor cells (EPCs) in neoangiogenesis and as potential candidates for restorative therapy in patients with IS. Silencing hypoxia-inducible factor prolyl hydroxylase 2 (PHD2) enhances the migration and survival of EPCs, thereby improving their therapeutic efficacy for IS [91].

Building on this knowledge, Wang *et al.* [92] successfully devised an innovative approach utilizing amphiphilic low-molecular weight polyethyleneimine (alkyl PEI)-encapsulated superparamagnetic iron oxide NPs for siRNA delivery. This system facilitates siRNA delivery and allows for the MRI-mediated tracking of EPCs. Silencing PHD2 in EPCs leads to increased CXCR4 and HIF-1α expression, thereby enhancing EPC migration and survival, respectively. The significant vascular-regenerative capacity of this approach was demonstrated based on the reduced infarct volume and improved functional deficits in a mouse model of stroke.

3.2.2. Active targeting drug delivery system

While previous studies have demonstrated increased BBB permeability during IS, it still poses a challenge to efficient drug delivery. In addition to the increased permeability, Ischemia induces endothelial cells to express specific receptors, providing an opportunity for nanoparticles to penetrate the BBB. Active targeting strategies include attaching ligands to the surface of NPs, facilitating their uptake through specific BBB receptors. This approach enhances passive extravasation, improving the delivery of NPs to the desired site within the brain. By actively targeting these receptors, ligand-functionalized NPs have the potential to improve the penetration and distribution of therapeutic agents, thereby enhancing the effectiveness of drug delivery across the BBB in IS treatment.

Upregulation of the expression of integrin $\alpha v\beta 3$ [93] on stressed vascular endothelial cells during cerebral ischemia and its interaction with the specific peptide ligand c(RGDyK) has been observed [94]. This interaction facilitates the infiltration of the ligand into the ischemic lesion. Building on this finding, Lu *et al.* [95] designed and developed a nanocarrier called an RNS-stimulated responsive liposome loaded with the NF-κB inhibitor caffeic acid phenethyl ester (CAPE) and coupled it with the c(RGDyK) peptide. This nanocarrier, known as r-lipoo-CAPE, was designed to actively target ischemic lesions and reduce cerebral ischemia/reperfusion injury by modulating neurovascular regeneration. Wang *et al.* [96] conducted a study wherein they synergistically integrated the thrombolytic activity of a functional peptide with the ROS scavenging capacity of a nanoenzyme. Through the utilization of a novel peptide-induced active targeting mechanism, the designed nanase successfully bound to fibrin within thrombi and efficiently traversed the BBB. Moreover, the combined nanoenzyme and peptide formulation, PNzyme/MnO_2, exhibited a prolonged blood circulation time, demonstrated a robust thrombolytic effect, and effectively mitigated ischemic damage in brain tissue, as observed in mouse and rat models of IS (Fig. 3).

The NF-κB signaling pathway assumes a pivotal role in the development of ischemia-reperfusion injury. A compound known as CAPE has demonstrated the capability to inhibit NF-κB and subsequently diminish neuroinflammation in cases of IS. CAPE induces a shift in microglial polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, thereby attenuating the inflammatory response. Additionally, CAPE aids in the reduction of damaged BBB infiltration. Moreover, nanocarriers can incorporate ROS as switch molecules that respond to specific pathological conditions in the ischemia/reperfusion zone of the brain. This enables controlled release of the loaded drug, targeting the affected area more effectively. In an *in vivo* study using a MCAO model, mice treated with r-lipoo-CAPE (CAPE-loaded nanocarriers) exhibited minimal neurological damage and reduced brain tissue damage. The infarct area was only 13% in the r-lipoo-CAPE-treated group, compared to 53% in the saline-treated group and 38% in the group treated with free CAPE. Additionally, microglia in the ischemic brain showed polarization towards a tissue-repairing M2 phenotype after treatment with r-lipoo-CAPE, indicating its potential for promoting neuroprotection and tissue repair in IS.

Mesenchymal stem cells (MSCs) for IS treatment has also garnered significant interest. However, the limited efficiency of MSC delivery to stroke lesions hinders their clinical application [97,98]. The study conducted by Kim *et al.* [99] revealed a notable finding wherein magnetic nanovesicles (MNVs) derived from MSCs were

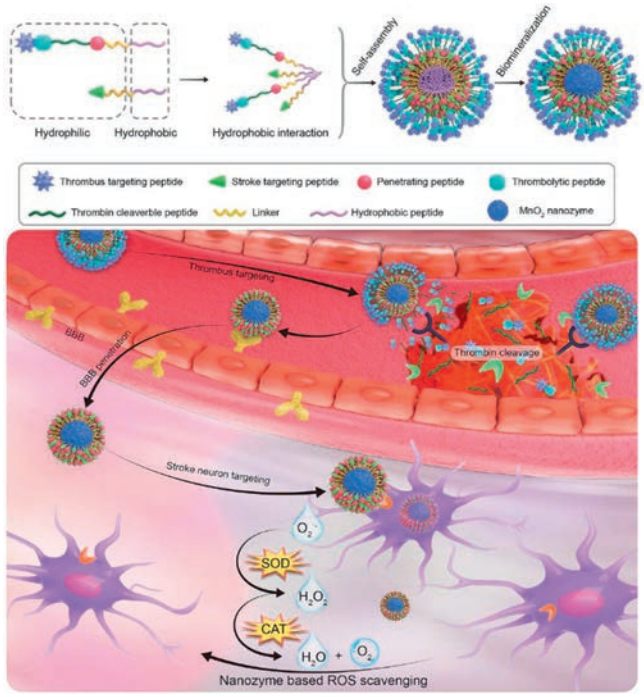

Fig. 3. Active targeted thrombolysis strategy; active thrombolysis across the BBB through self-assembly of thrombus-targeting and thrombolysis-targeting peptides with hydrophobic peptides. Reproduced with permission [96]. Copyright 2023, John Wiley & Sons.

loaded with iron oxide nanoparticles (IONPs) to facilitate active targeting of the ischemic site utilizing an external magnetic field. This targeted strategy effectively enhances the delivery of therapeutic agents to the ischemic lesions, thereby significantly improving their therapeutic efficacy.

In comparison to nanovesicles derived from MSCs, the incorporation of IONPs in MSC-IONPs exhibited a notable enhancement in the expression of therapeutic growth factors. This upregulation was attributed to the stimulation of c-Jun phosphorylation and subsequent activation of c-Jun N-terminal kinase signaling molecules within MSCs. In a rat model of MCAO, the intravenous administration of MNVs led to a remarkable 5.1-fold increase in their ability to specifically target ischemic lesions. Moreover, MNV treatment demonstrated the promotion of anti-inflammatory responses, angiogenesis, and apoptosis in the context of ischemic brain injury, consequently resulting in a significant reduction in infarct volume and notable improvements in motor function [100,101].

In recent years, the return of bone marrow-derived MSCs to the damaged brain has remained a challenge for effective treatment. Clinical translation using liposome-mediated nano-delivery systems has some challenges and limitations. First, the target effect is low. Second, the long-term-exposure toxicity, genotoxicity, and developmental and reproductive toxicity of NPs, which are important factors influencing the clinical translation of nanomedicines, have not yet been systematically studied.

3.3. Advanced drug delivery for anti-oxidative effects

Oxidative stress is a prominent response following an ischemic attack and is characterized by excessive ROS production. An insufficient antioxidant defense coupled with blood reperfusion can lead to the accumulation of ROS, including superoxide anions $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$, during ischemia [102]. This accumulation further contributes to microvascular dysfunction and oxidative damage, triggering cell death mechanisms, such as apoptosis, and disrupting the BBB [103]. Intracellular redox homeostasis is normally maintained by various antioxidant enzymes, such as SOD and CAT. However, highly elevated levels of $O_2^{\bullet-}$ overwhelm these enzymes, making it challenging to adequately replenish them during disease progression [104,105]. Additionally, $O_2^{\bullet-}$ can react with other molecules (*e.g.*, H_2O_2 and -NO) to generate secondary reactive oxygen and nitrogen species (RONS), including $^{\bullet}OH$ and ONOO-. These secondary RONS are highly reactive and significantly contribute to oxidative damage, yet there are no specific enzymes targeting them. The overproduced RONS can act as signaling molecules, triggering microglial activation, peripheral leukocyte infiltration, and cytokine secretion by inflammatory cells [106]. Furthermore, the excessive accumulation of RONS can lead to the inactivation and depletion of endogenous antioxidant enzymes, resulting in oxidative damage in brain regions, particularly in the ischemic hemispheric zone, which represents a potentially salvageable area of the ischemic brain [71]. Therefore, the design of nanomedicines for neuroprotection in IS regions should focus on the elimination of ROS, aiming to restore the redox balance and mitigate oxidative damage (Table S3 in Supporting information).

3.3.1. Endogenous anti-oxidase enzyme-loaded NPs

Endogenous antioxidant enzymes, including melanin, glutathione peroxidase (GSHPx), CAT, and SOD, have demonstrated excellent biocompatibility and potent antioxidant activity, showing promise for IS treatment. Melanin possesses inherent free radical-scavenging abilities and exhibits antioxidant properties. Liu *et al.* [106] prepared PEG-stabilized melanin NPs (MeNPs) under physiological conditions by coupling amine-capped PEG to the surface of MeNPs. Researchers have also extensively investigated the effects of MeNPs on various RONS, including $O_2^{\bullet-}$, -NO, H_2O_2, $^{\bullet}OH$, and ONOO-, which are major toxic species implicated in disease processes. *In vitro* and *in vivo* evaluations were conducted to assess the antioxidant therapeutic potential of MeNPs in the context of IS. The study revealed that MeNPs exert broad protective effects against the aforementioned RONs, effectively attenuating the associated inflammatory response by inhibiting the expression of inflammatory mediators and cytokines. Furthermore, in comparison to natural antioxidant enzymes, such as SOD, and other nanoantioxidants targeting specific RONs that are currently being investigated, PEG-MeNPs demonstrated unique properties, including multiple antioxidant and anti-inflammatory effects, as well as enhanced biocompatibility. These findings highlight the potential of using MeNPs as a promising approach for the treatment of IS, offering advantages over existing antioxidant enzyme-based strategies and targeted nanoantioxidants.

Copper/zinc superoxide dismutase (CuZnSOD or SOD1) exhibits potential as a therapeutic candidate for conditions linked to oxidative stress. However, its clinical application is impeded by its short half-life and limited ability to cross BBB. In order to overcome these limitations, it is necessary to develop strategies and interventions that specifically address these challenges. Jiang *et al.* [107] developed a novel NP-based approach to enhance the loading efficiency and immediate availability of CAT and SOD1. The researchers incorporated SOD1 into a core–shell-structured NP, where the core consisted of charge-neutralized polycationic chains and protein spheres and the shell was composed of PEG. A low-molecular-weight chemical crosslinker was employed to create a crosslinked (cl) core to form the cl-nanoenzyme. This formulation, referred to as SOD1 (cl-nanase), has several advantages over PEGylated SOD1. The cl-nanase formulation demonstrated the passive targeting of damaged cerebral blood vessels, resulting in superior intracellular ROS depletion and the inhibition of angiotensin II signaling both *in vitro* and *in vivo*. When administered intravenously to rats with MCAO, the nanoformulated SOD1 actively bound the developing thrombi that occurred during or after stroke. This led

to a reduced infarct volume and improved sensorimotor function in MCAO-model rats. These findings highlight the potential of the clnanase formulation as an effective strategy for targeted SOD1 delivery, enabling enhanced therapeutic outcomes in IS by mitigating ROS-mediated damage and influencing angiotensin II signaling.

3.3.2. Inorganic nano-reductase-based NPs

Inorganic nanomaterials have received considerable attention for IS treatment owing to their excellent thermal, optical, magnetic, and catalytic properties. Recently, the powerful enzymatic antioxidant activities of several inorganic nanomaterials, such as cerium NPs and Mn_3O_4 nanozymes, have been discovered. Cerium oxide nanoparticles (CeO_2 NPs) have emerged as promising free-radical scavengers with potent antioxidant effects, making them excellent carriers for therapeutic applications. In the context of neuroprotective therapy following ischemia-reperfusion injury, Li *et al.* [108] developed a strategy combining vascular regeneration and antioxidant approaches using NBP-loaded CeO_2 NPs (NBP-CeO_2 NPs). NBP is a neuroprotective drug known for its vascular-regenerative properties, including improved microcirculation in ischemic areas, the promotion of angiogenesis, neurogenesis, and neuroplasticity, the inhibition of inflammation, and the suppression of apoptosis. By encapsulating NBP within PEGylated CeO_2 NPs, NBP-CeO_2 NPs facilitated a synergistic treatment approach for IS.

The findings from this study provide compelling evidence supporting the efficacy of NBP-CeO_2 nanoparticles in scavenging ROS in brain microvascular endothelial cells and hippocampal neurons during OGD/R. These nanoparticles demonstrate the ability to preserve the mitochondrial membrane potential, morphology, and function, ultimately resulting in the mitigation of BBB disruption and neuronal apoptosis. In a mouse model of MCAO, NBP-CeO_2 nanoparticles exhibit remarkable ROS-scavenging capabilities, effectively preserving BBB integrity, and reducing brain infarction, brain edema, neuroinflammation, and neuronal apoptosis. Furthermore, long-term neurobehavioral assessments in rats reveal that the administration of NBP-CeO_2 nanoparticles significantly improves sensorimotor functions and spatial learning by promoting angiogenesis after IS. These findings highlight the therapeutic potential of NBP-CeO_2 nanoparticles as a comprehensive treatment approach for IS, effectively targeting ROS-mediated damage, preserving BBB integrity, and promoting neuroregeneration.

Mn_3O_4 nanomaterials have garnered considerable interest in the field of addressing ischemia–reperfusion injuries due to their small size and noteworthy enzyme-mimicking characteristics. These nanomaterials possess the ability to release manganese ions into circulation, consequently promoting the activity of SOD *in vivo*. SOD assumes a critical role in providing short- and long-term neuroprotection. In order to enhance the stability of Mn_3O_4 nanomaterials in aqueous environments, various strategies have been explored. Huang *et al.* [109] employed human serum albumin for the surface modification of Mn_3O_4 and developed a translational Mn_3O_4 nanoenzyme called HSA-Mn_3O_4. HSA-Mn_3O_4 exhibits a prolonged circulation time in the bloodstream owing to the stabilizing effect of the protein. Additionally, surface modification improved its anti-inflammatory and ROS-scavenging enzyme-mimicking abilities. HSA-Mn_3O_4 could inhibit neurological damage by OGD, OGD-mediated apoptosis, and endoplasmic reticulum stress in SH-SY5Y and primary neuronal cells. These findings further show the enhanced antioxidant neuroprotective capacity of HSA-Mn_3O_4 against IS and brain tissue reperfusion injury. Overall, Mn_3O_4 nanomaterials, particularly the HSA-Mn_3O_4 nanoenzyme, hold promise as effective agents for combating ischemia–reperfusion injury because of their enzyme-mimicking properties, prolonged circulation time, improved anti-inflammatory capabilities, and ROS-scavenging abilities (Fig. 4).

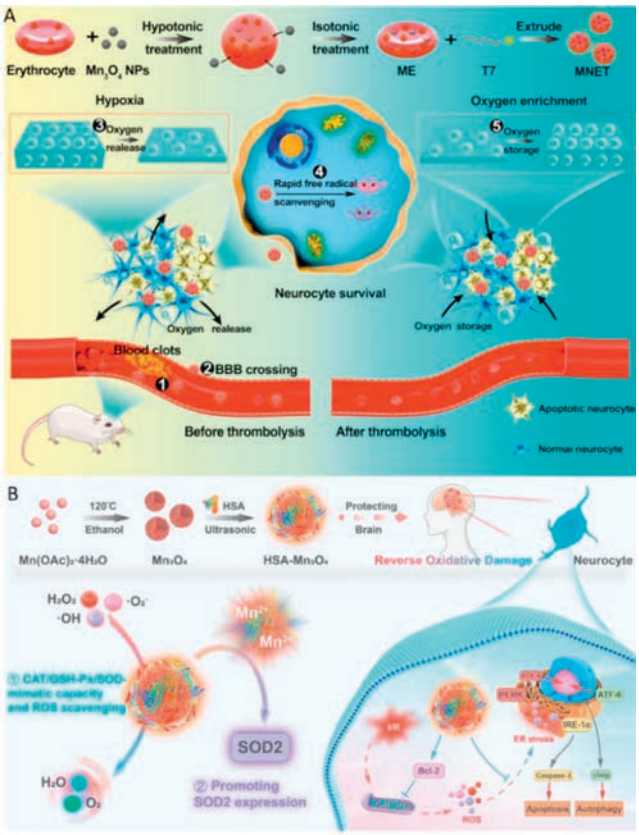

Fig. 4. Advanced drug delivery for anti-oxidative effects. (A) Schematic representation of protein-stabilized multifunctional Mn_3O_4 nano-enzymes with HSA surface modification for enhanced biocompatibility of Mn_3O_4 for mimicking antioxidant enzymes and scavenging of excess ROS to attenuate oxidative stress and apoptosis induced by reperfusion injury in IS. Reproduced with permission [109]. Copyright 2022, American Chemical Society. (B) Engineered nano-erythrocytes to treat acute IS and ischemia/reperfusion injury continuously. Mn_3O_4 nanoenzymes are encapsulated in natural red blood cells, which are modified by T7 peptide to confer BBB penetration ability, and exhibit nano-sponge *in vivo* to absorb oxygen and release oxygen at the lesion to achieve effective treatment of IS. Reproduced with permission [110]. Copyright 2020, American Chemical Society.

3.3.3. Anti-oxidase drug-loaded NPs

EDV and succinylcholine (SCB) are antioxidant drugs commonly used to treat IS because they scavenge ROS. EDV is a small ROS scavenger that exerts neuroprotective effects. To enhance its brain uptake and effectiveness, Jin *et al.* [111] developed an agonist micelle formulation called EDV-AM. In this formulation, EDV was encapsulated within the inner core of PEG-PLA micelles *via* hydrophobic interactions. The micelles were further modified with an adenosine 2A receptor (A2AR) agonist and the hepta-methylpurinocyanine derivative IR783B fluorophore, creating a hydrophilic shell. These micelles were found to regulate BBB permeability by targeting A2AR. After stroke, A2AR expression is upregulated in the brain capillaries within the ischemic region. The EDV-AM formulation, through A2AR signaling, triggers the opening of active TJs, specifically in ischemic vessels. This enables the targeted delivery of EDV to the ischemic region. Once released, this drug efficiently clears the ROS produced by brain cells and infiltrating inflammatory cells, such as macrophages, neutrophils, and T cells, thereby exerting neuroprotective effects. Both high-performance liquid chromatography and MRI studies have demonstrated that EDV-AM has a greater ability to rescue ischemic tissue than free EDV. Additionally, diffusion tensor imaging has shown that EDV-AM is effective in promoting the remodeling of white matter axons and improving functional behavior in models of IS.

Overall, the EDV-AM formulation, with its A2AR-targeting capability and enhanced neuroprotective effects, holds promise for the treatment of IS, *via* effective ROS scavenging, inflammatory cell clearance, and the promotion of tissue repair and functional recovery.

He *et al.* [72] developed a bionanoparticle platform combining pH-targeting and BBB-targeting strategies to enhance the penetration of NPs through the BBB to reach the site of brain injury. This platform involved loading SCB into an amphiphilic pH-sensitive polymer called methoxypolyethylene glycol-*block*-poly(2-isopropyl methacrylate) and camouflaging the NPs with 4T1 cancer cell membranes, resulting in MPP/SCB NPs. The 4T1 cancer cell membranes on MPP/SCB NPs contain syndecan-1 (CD138), which can bind to platelet endothelial cell adhesion molecule-1 (CD31) and vascular cell adhesion molecule 1 (VCAM1). These adhesion molecules are overexpressed in perivascular regions of the brain. The MPP/SCB NPs encapsulated in 4T1 cell membranes were found to preferentially adhere to the stroke-affected site in a bionic manner and were internalized by various brain cells.

Once inside the cells, the NPs respond to the acidic intracellular environment, leading to SCB release and its subsequent pharmacological effects. After intravenous injection of the nanoformulation, MPP/SCB demonstrated good targeting of the ischemic hemisphere and exerted neuroprotective effects. The MPP/SCB delivery rate to the ischemic hemisphere was 4.79-fold higher than that to the normal hemisphere, resulting in a significant reduction in the infarct volume of 69.9%. This bionanoparticle platform, developed by He *et al.*, leverages pH- and BBB-targeting strategies, along with the use of cancer cell membranes, to enhance the delivery of SCB to the site of the brain injury in IS. This targeted delivery and SCB release leads to neuroprotection and a reduction in the infarct volume, showing promise for IS treatment.

Nanodelivery systems have emerged as promising strategies to overcome the limitations associated with antioxidant drugs used in the treatment of IS, such as poor water solubility and extended half-life. These systems possess the potential to enhance drug penetration across the BBB and thereby improve therapeutic outcomes. However, it is essential to carefully evaluate the potential health effects related to the use of exogenous nanomaterials in such systems. Adverse effects that need to be considered include oxidative stress generation, activation of immune cells, disturbances in mitochondrial respiration, and genotoxicity, as these factors could impact the *in vivo* applications of nanodelivered drugs. Therefore, it is imperative to conduct comprehensive investigations to assess the biocompatibility and cytotoxicity of these nanodelivery systems *in vivo*, ensuring their safe and effective utilization for IS treatment [112].

Furthermore, a design strategy involving the binding of targeted ligands to nanocarriers for specific delivery poses challenges, including complex dosing methods and difficulties in achieving a controlled dosage. Factors such as the integrity of the nanocarrier, ligand-binding efficiency, and ability to penetrate the BBB must be meticulously examined and optimized to ensure efficient and reliable drug delivery. In conclusion, whereas nanodelivery systems have considerable potential for improving the therapeutic efficacy of antioxidant drugs for IS treatment, rigorous scientific investigations are imperative to address safety concerns, optimize their design, and establish controlled dosing strategies, ultimately providing a foundation for their successful clinical application.

4. Future perspectives and conclusions

In this comprehensive review, we provide an overview of advanced neuroprotectant-based nano-drug delivery systems for the treatment of IS. Whereas neuroprotective agents have demonstrated promising potential for IS therapy and some drugs have been approved for clinical use, the limitations associated with their standalone administration necessitate the development of effective solutions, and nanodrug delivery systems offer a promising approach to address this [113]. The BBB presents a significant obstacle to the delivery of drugs into the brain parenchyma, particularly in the context of treating conditions like IS. This barrier restricts the passage of many substances, complicating drug delivery efforts. There are various pathways that can facilitate the movement of molecules across the BBB. These include receptor- and carrier-mediated pathways, which are particularly effective for lipophilic substances. However, the focus of many nanodrug delivery systems has primarily been on achieving brain-specific targeting, often neglecting the equally important issue of drug distribution within the brain parenchyma. While some promising targeting ligands like RVG29 and the stroke homing peptide SHp have been identified, they encounter challenges similar to those faced by receptor-mediated transcytosis strategies. Various modes of nanodelivery have been explored to overcome these challenges. These include transcytophilic lipophilic transport, physically-assisted transport, carrier-mediated transport, receptor-mediated transport, nasal delivery, and biomimetic drug delivery. However, despite these strategies, challenges related to delivery efficiency and mode of administration persist. Often, the accumulation of drugs in other organs, such as the liver and kidneys, can overshadow the intended brain targeting. Therefore, it is crucial to systematically investigate *in vivo* drug metabolism to ensure that the nanodelivery system maintains an effective drug concentration after crossing the BBB. One innovative approach is nasal drug delivery, which holds the potential to address delivery efficiency concerns. However, the translation of this method to clinical use faces hurdles, including the development of mature drug delivery devices and the risk of drug loss due to normal physiological functions like respiration. Conventional drug delivery methods such as oral and tail vein injections also encounter their own set of challenges. The choice of delivery method depends on factors such as the drug's molecular size, polarity, and lipid solubility. While oral administration may seem gentle, many nanodelivery systems struggle to successfully transport drugs across the BBB through this route. Most nanodelivery systems are injected *via* the tail vein. With the incorporation of targeted peptides, bionic systems, and lipids, it is possible to enhance BBB penetration, reduce systemic toxicity, and maintain an effective drug concentration after crossing the BBB. However, it is crucial to consider whether the accumulation of drugs in other organs might trigger organ toxicity during the design phase of the nano-drug delivery system. In conclusion, delivering drugs across the BBB is a complex challenge with multiple strategies available, but it requires careful consideration of various factors. Addressing issues related to delivery efficiency, administration modes, and potential organ toxicity is essential to advance drug delivery systems for conditions like IS.

In recent years, there have been notable advancements in the treatment of IS, with the emergence of three promising therapeutic strategies: the reduction of excess ROS, the modulation of inflammation levels, and the promotion of neuronal regeneration. These strategies offer the potential to improve outcomes for IS patients. Moreover, the integration of advanced nanomedicine delivery systems into these approaches has shown great promise. These systems can produce synergistic effects that substantially enhance brain protection against IS, further underscoring their potential as valuable tools in the fight against this debilitating condition. However, despite extensive research in this field, no nanodrug specifically designed for IS treatment has been successfully transitioned from the laboratory to clinical practice. Several key challenges must be addressed before this clinical translation can be achieved. (1) Preparation process, scaling-up production and establishing uniform quality standards pose significant obstacles. For

example, ensuring stable drug-loading and encapsulation rates of liposomes remains a challenge, as current methods are primarily limited to laboratory-scale production [114]. At the same time, the large-scale preparation of large quantities of nanomedicines also faces many technical challenges. At present, the preparation of nanomedicines in the laboratory often faces problems, such as cumbersome preparation process, different batches of prepared products and small amount of preparation. How to further expand the scale of nanomedicine production and the quality of the production is still a difficult problem in clinical translation. (2) Nanosafety, the safety and reliability of nano-delivery systems is the most important concern for their clinical translation, most brain-targeting drug delivery systems are complex, and a comprehensive understanding of the *in vivo* circulating metabolic processes in these systems is lacking. It is also important to investigate whether the protein corona formed upon entry into the body, which is cleared by the immune system and bound to plasma proteins, affects the activity, targeting efficiency, and potential off-target effects of these delivery systems. The absorption, distribution, and release of nanocarriers need to be fully investigated before clinical translation; in addition, the material toxicity of the nanodelivery system itself still needs to be considered, especially the use of inorganic metals, extracellular vesicles, and liposomes as drug carriers [115]. (3) Animal models, the choice of the disease model used in preclinical studies presents another hurdle. Although rat and rabbit models are commonly used to simulate human conditions, the heterogeneity between species and the presence of multiple comorbidities in most patients with brain diseases make it challenging to accurately evaluate the efficacy of existing animal models in terms of predicting human outcomes. Thus, further in-depth studies are required to assess drug efficacy, metabolism, and toxicity to achieve efficient drug delivery across the BBB. (4) Nanostability, attention must be focused on the stability of nano-drug delivery systems. Many of these systems require strict production and storage conditions to maintain stability, and their long-term stability over extended periods requires thorough investigations [116]. (5) Targeting efficiency, whether the brain-targeted drug delivery system can achieve effective controlled release of drugs through the BBB is the key to achieve disease treatment, and the targeting efficiency directly affects the drug efficacy; for example, through the modification of targeted peptides, targeting the specific receptors on the brain microvascular endothelial cells, or the use of endogenous biomimetic carriers and extracellular vesicles to realize the drug delivery, however, most of the experiments at present only consider whether the nanoparticle delivery system can pass the BBB, some inorganic nanoparticles can be simply quantified by ICP-MS, however, the vast majority of drug delivery systems are unable to accurately consider the targeting efficiency, therefore, a complete set of targeting efficiency evaluation system should be improved as soon as possible. ADDSs face numerous obstacles before being successfully translated into clinical practice. Addressing the challenges related to production scale-up, quality standards, *in vivo* metabolism, disease model choices, and long-term stability is crucial for advancing the clinical applications of these systems. Even with many challenges, development of new carriers and technological advances, neuroprotection-based nano-delivery systems show promise for the treatment of IS. For example, surface modification technology, coating technology, and cross-linking technology [117] have modified the nano-delivered drugs to enhance their stability. In addition, novel precursors, such as metal-organic frameworks, and novel solvents, such as supercritical fluids, have been introduced to help the large-scale production of nanodrugs [118].

In summary, this comprehensive review presents an in-depth overview of advanced neuroprotectant-based drug delivery systems for IS, highlighting their biomedical applications and potential to revolutionize targeted therapy for this condition. Considering the intricate pathological mechanisms associated with IS, anti-inflammatory, antioxidative, and revascularization strategies, which are crucial for effective IS treatment, have been incorporated into these drug delivery systems. This review also discusses the various targeting mechanisms employed by novel nanocarriers and proposes their potential mechanisms of action. Additionally, the review addresses the need for studies on the sustainability of neuroprotectant-based brain-targeting drug delivery systems and provides insights into the challenges and limitations associated with their clinical translation. The aim of these discussions is ultimately to facilitate the successful clinical application of nanomedicines for the treatment of IS.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109149.

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