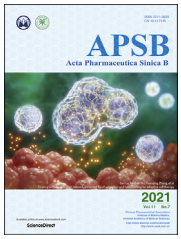
Acta Pharmaceutica Sinica B 2021;11(7):1767−1788

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Recent advances in nanomedicines for the treatment of ischemic stroke

Chao Li, Tao Sun*, Chen Jiang*

Key Laboratory of Smart Drug Delivery, Ministry of Education, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Department of Pharmaceutics, School of Pharmacy, Research Center on Aging and Medicine, Fudan University, Shanghai 201203, China

Received 28 June 2020; received in revised form 27 August 2020; accepted 13 September 2020

KEY WORDS

Stroke;
Ischemic cascade;
Reperfusion;
Neuroprotectant;
Thrombolytics;
Nanomedicine;
Blood–brain barrier

Abstract Ischemic stroke is a cerebrovascular disease normally caused by interrupted blood supply to the brain. Ischemia would initiate the cascade reaction consisted of multiple biochemical events in the damaged areas of the brain, where the ischemic cascade eventually leads to cell death and brain infarction. Extensive researches focusing on different stages of the cascade reaction have been conducted with the aim of curing ischemic stroke. However, traditional treatment methods based on antithrombotic therapy and neuroprotective therapy are greatly limited for their poor safety and treatment efficacy. Nanomedicine provides new possibilities for treating stroke as they could improve the pharmacokinetic behavior of drugs *in vivo*, achieve effective drug accumulation at the target site, enhance the therapeutic effect and meanwhile reduce the side effect. In this review, we comprehensively describe the pathophysiology of stroke, traditional treatment strategies and emerging nanomedicines, summarize the barriers and methods for transporting nanomedicine to the lesions, and illustrate the latest progress of nanomedicine in treating

Abbreviations: AEPO, asialo-erythropoietin; APOE, apolipoprotein E; BBB, blood–brain barrier; BCECs, brain capillary endothelial cells; CAT, catalase; Ce-NPs, ceria nanoparticles; COX-1, cyclooxygenase-1; cRGD, cyclic Arg-Gly-Asp; CsA, cyclosporine A; CXCR-4, C-X-C chemokine receptor type 4; DAMPs, damage-associated molecular patterns; e-PAM-R, arginine-poly-amidoamine ester; GFs, growth factors; GPIIb/IIIa, glycoprotein IIb/IIIa; Hb, hemoglobin; HMGB1, high mobility group protein B1; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; LFA-1, lymphocyte function-associated antigen-1; LHb, liposomal Hb; MCAO, middle cerebral artery occlusion; miRNAs, microRNAs; MMPs, matrix metalloproteinases; MSC, mesenchymal stem cell; NF-κB, nuclear factor-κB; NGF, nerve growth factor; NMDAR, *N*-methyl-D-aspartate receptor; nNOS, neuron nitric oxide synthase; NOS, nitric oxide synthase; NPs, nanoparticles; NSCs, neural stem cells; PBCA, poly-butyl-cyanoacrylate; PCMS, poly (chloromethylstyrene); PEG, poly-ethylene-glycol; PEG-PLA, poly (ethylene-glycol)-*b*-poly (lactide); PLGA NPs, poly (L-lactide-*co*-glycolide) nanoparticles; PSD-95, postsynaptic density protein-95; PSGL-1, P-selectin glycoprotein ligand-1; RBCs, red blood cells; RES, reticuloendothelial system; RGD, Arg-Gly-Asp; ROS, reactive oxygen species; SDF-1, stromal cell-derived factor-1; SHp, stroke homing peptide; siRNA, small interfering RNA; SOD, superoxide dismutase; SUR1-TRPM4, sulfonylurea receptor 1-transient receptor potential melastatin-4; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; TIA, transient ischemic attack; TNF-α, tumor necrosis factor-α.

*Corresponding authors. Tel./fax: +86 21 5198 0187.

E-mail addresses: sunt@fudan.edu.cn (Tao Sun), jiangchen@shmu.edu.cn (Chen Jiang).

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2020.11.019

ischemic stroke, with a view to providing a new feasible path for the treatment of cerebral ischemia.

1. Introduction

Stroke is the second leading cause of death and severely affects the daily life of survivals due to its high disability rate[1]. Ischemic stroke normally occurs when a vessel that supplies blood to the brain is blocked, and it makes up approximately 87% of all stroke cases[2]. Ischemia would initiate the ischemic cascade which involves a series of biochemical events, including energy failure[3], ion imbalance and excitotoxicity[4], oxidative stress[5–7], cell death (apoptosis or necrosis), activation of the complement system[8–12], initiation of the inflammation and immune response. The cascade would eventually lead to irreversible brain damage[13]. Many strategies have been adopted to reconstruct blood supply to the brain, aiming to address the aforementioned harmful effects of ischemia[14]. Thrombolysis with tissue plasminogen activator to realize recanalization to the brain is the gold standard for ischemic stroke treatment. However, the time window of thrombolysis is relatively narrow (≤4.5 h), due to the risk of hemorrhagic transformation (HT) caused by the low targeting ability to thrombus[15,16]. Thus, only a small number of patients could receive and benefit from the thrombolytic therapy in time. Moreover, restoring blood flow to the ischemic brain would cause secondary reperfusion injury. Reperfusion would exacerbate the production of reactive oxygen species (ROS) and the amplification of inflammation and immune response, which would cause undue neural death, impair the integrity of blood–brain barrier (BBB) and eventually lead to brain edema[17–20]. There are also numerous preclinical studies focusing on protecting neurons from the injury at different stages of ischemia and reperfusion. However, most neuroprotective agents have unneglectable defects including low solubility, short half-life and poor BBB permeability *in vivo*, which might contribute to the failure of many clinical trials[21,22]. It is difficult and costly to find safer and more effective new drugs to treat ischemic stroke. Thus, there is an increasing demand to endow them with pharmaceutical improvement to existing drugs to suit the clinical requirements.

Numerous nanomedicines have been developed to improve the efficacy and to reduce the side effects of traditional treatment methods, since well-designed nanomedicines possess many unique advantages. First, nanomedicine could increase the solubility of poorly soluble drugs, improve the stability and extend the half-lives of drugs *in vivo*[23]. Second, targeted modified nanomedicine could assist drugs to cross the BBB which is a main barrier for most drugs to reach the brain, and realize accumulation at the desired site to avoid non-specific distribution. Targeted modification could also help the nanomedicine to mainly taken up by specific cells, such as damaged neurons. Hence, nanomedicines with targeting ability could not only increase the therapeutic effect, but also reduce the injected dose and toxicity of drugs[24]. In addition, nanomedicines consisted of different functional materials could control the release of drugs, and the functionalized vehicle might be effective in treating ischemic stroke[23]. Moreover, nanomedicines provide possibilities for some emerging treatments, such as gene therapy, which is favor of recovering from injury at the chronic phase of stroke. Nanomedicines include liposomes, micelles, nanoparticles (NPs), emerging cell membrane coated NPs and exosomes, all of which have been applied in the preclinical studies for treating ischemic stroke. Apart from discussing the pathophysiology of ischemic stroke which might affect the design of nanomedicine, as well as traditional treatment strategies and their deficiencies, this review mainly focuses on investigating the barriers and methods to transporting nanomedicines to the targeting sites and summarizes preclinical studies of nanomedicines for the treatment of ischemic stroke according to various abnormalities at different phases of ischemic cascade.

2. The pathophysiology of ischemic stroke

Understanding the pathological mechanisms of ischemic stroke is helpful in finding therapeutic targets and developing more efficiently targeted nanomedicines. A series of molecular and cellular events are involved in the ischemic cascade, which occur at different time and interconnect with each other. In the ischemic core, hypoperfusion restricts the oxygen/glucose supply to the brain, which causes the energy failure of neurons[3]. Insufficient ATP causes the ion pumps to operate abnormally. As a result, the ion gradient across the cell membrane is out-of-balance[13]. Increased influx of Na^+ and efflux of K^+ leads to the depolarization of neurons and glial cells, as well as extracellular accumulation of glutamate that can cause excitotoxicity[4]. Intracellular accumulated Na^+ and following increased Cl^- will result in the swelling of cells, destruction of cell membrane and final necrosis. In the ischemic penumbra, extracellular accumulated glutamate over-activates the glutamate receptors, which leads to the influx of Ca^{2+} [13]. Then numerous Ca^{2+}-dependent enzymes are activated, including proteases which destroy the cell integrity, nitric oxide synthase (NOS) and enzymes producing ROS[5,13]. ROS not only directly reacts with macromolecules to cause lipid peroxidation, protein oxidation and DNA damage, but also causes indirect injury through multiple signaling pathways, such as caspase-mediated apoptosis[6,7].

Reperfusion would exacerbate the production of ROS and subsequent activation of matrix metalloproteinases (MMPs), which would worsen the damage of neurovascular units[17–20,25,26]. Neurons under stress and necrotic cells would release damage-associated molecular patterns (DAMPs), such as high mobility group protein B1 (HMGB1) and ATP, which would initiate the recruitment of intrinsic microglia and upregulate the expression of pro-inflammatory mediators[27–30]. Pro-inflammatory mediators such as chemokines and adhesion molecules would drive and promote the infiltration of neutrophils and blood-borne

macrophages into the ischemic brain[31–35]. Complement system can be activated in the blood and brain parenchyma as well, which leads to increased leukocytes infiltration[8–12]. Neutrophils and mononuclear phagocytes recruited to the ischemic brain will contribute to the production of inducible nitric oxide synthase (iNOS), ROS, MMPs and other inflammatory mediators such tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), which eventually impairs the BBB permeability and leads to brain edema[36,37]. The multiple molecular and cellular events that involved in the ischemic cascade are illustrated in Fig. 1.

3. Traditional treatment strategies

Conventional therapy for ischemic stroke mainly includes the employment of antithrombotic drugs to improve the brain blood circulation, and neuroprotectant to protect neurons from death induced by ischemia and reperfusion.

3.1. *Antithrombotic drugs*

Clinically used antithrombotic drugs are generally classified into antiplatelet and anticoagulant drugs, which are applied to prevent thrombosis, and thrombolytic drugs to dissolve the existed thrombus[38]. Platelets play a vital role in the formation of thrombus, and antiplatelet treatment not only prevents the formation of thrombus, but also reduces the growth of thrombus[39]. For instance, aspirin is the most widely used drug for antiplatelet treatment, as aspirin could selectively inhibit the cyclooxygenase-1 (COX-1) involved in the platelet adhesion at a low dosage without inhibiting the synthesis of prostacyclin[40]. Anticoagulants such as heparins, vitamin K antagonists and argatroban have also been applied for treating ischemic stroke, as they could reduce the formation of secondary blood clots to improve prognosis. In detail, heparins could bind to antithrombin to deactivate thrombin and inhibit clotting factors; vitamin K antagonists, such as warfarin would reduce the production of activated clotting factors; argatroban could directly inhibit thrombin to inhibit the formation of blood clot[41].

Being different from antiplatelet and anticoagulant drugs, thrombolytic agents could activate plasminogen to form plasmin to degrade the fibrin involved in the thrombus, dissolve the formed clot and restore the blood supply to the ischemic brain. Urokinase and alteplase are inherent plasminogen activators *in vivo*. As alteplase could initiate the fibrin-specific fibrinolytic process, it is more widely applied clinically than urokinase. Many new plasminogen activators with improved fibrin specificity and prolonged half-life have been developed, such as desmoteplase and tenecteplase[42–44].

3.2. *Neuroprotective agents*

Neuroprotective agents could act on different pathophysiological pathways involved in ischemic cascade, and thus exert neuroprotective effects at different stages of stroke. One main

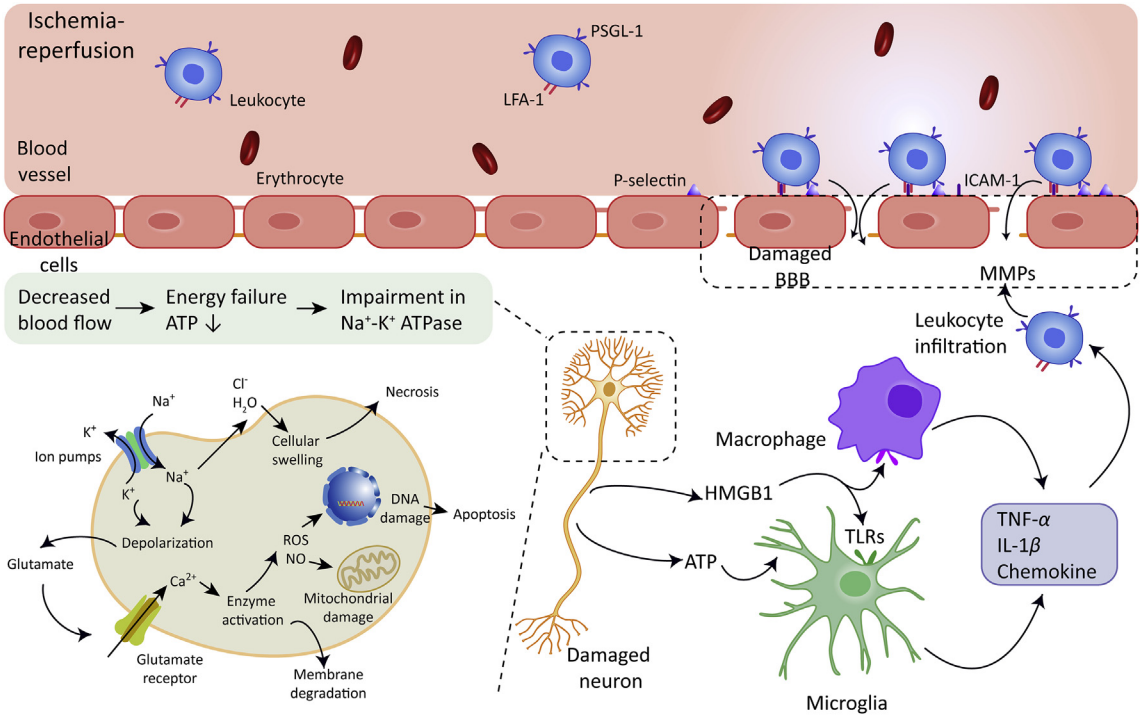


Figure 1 Molecular and cellular events involved in the ischemic cascade, including energy failure, ion imbalance and excitotoxicity, oxidative stress, cell death (apoptosis or necrosis), initiation of the inflammation and immune response. The expression of P-selectin and intercellular adhesion molecule-1 (ICAM-1) on brain capillary endothelial cells (BCECs) are up-regulated during stroke. The interaction between P-selection and P-selectin glycoprotein ligand-1 (PSGL-1) which are expressed on leukocytes promotes the binding of leukocytes on BCECs. Leukocytes realized firmer adhesion on the vascular wall through the specific binding between the ICAM-1 and lymphocyte function associated antigen-1 (LFA-1), leukocytes subsequently infiltrate into the ischemic brain parenchyma through the damaged blood–brain barrier (BBB). TLRs, Toll-like receptors.

therapeutic goal is to prevent glutamate-mediated excitotoxicity. Memantine, an *N*-methyl-D-aspartate receptor (NMDAR) antagonist, is able to reduce the lesion volume without interfering with the normal physiological function of NMDAR[45]. Another method to reduce the deleterious effects of excitotoxicity is regulating downstream signaling pathways activated by glutamate, such as the influx of Ca^{2+}. Nimodipine is a calcium channel blocker, and it could realize limited neuroprotective effects in preclinical models[46]. Eliminating oxidative stress is also a potential treatment strategy to rescue ischemic neurons. Edaravone is a free radical scavenger and able to inhibit lipid peroxidation, which has shown significant neuroprotective effect in clinical trials[47,48].

Moreover, current research mainly focused on attenuating the immune-mediated inflammatory response during the acute phase of ischemic stroke. Neutrophils play an important role in the occurrence and development of inflammation, treatment with antibody against E-selectin could reduce the infiltration of neutrophils into the ischemic brain and realize reduction in the infarct volume[49]. Fingolimod is an immunosuppressant that restricts lymphocytes migration from lymph nodes and reduces their infiltration into the brain. Administration of fingolimod could significantly reduce the infarct volume and decrease the BBB permeability, and improve the neurological function of patients[50]. The application of anti-inflammation agents and immunosuppressant reveals a new strategy for neuroprotection, yet the clinical application of neuroprotectant is still not optimistic. Table 1[40–42,45-50] summarizes the types of traditional treatment strategies, representative therapeutic agents and their molecular and cellular targets.

3.3. *Deficiencies of traditional treatment strategies*

Although some antithrombotic drugs and neuroprotectant have been used clinically, there are still some problems needed to be resolved to obtain safer and more effective therapeutic agents. For most antithrombotic drugs, the non-specific distribution and low thrombus targeting efficiency would increase the risk of bleeding in patients. Anticoagulant therapy could reduce the recurrence rate of ischemia during acute phage of stroke, but the benefit is often offset by the increase in symptomatic intracranial hemorrhage[41]. The effect of thrombolysis with plasminogen activator is also limited, since the circulation time of thrombolytic proteins is relatively short *in vivo* due to the clearance of proteinase, and the use of thrombolytics would increase the risk of bleeding. For instance, alteplase might amplify the role of MMPs while dissolving the thrombus, and thereby destroys the intercellular matrix of the BBB and increases the probability of intracerebral hemorrhage[51].

There are also some deficiencies in neuroprotective treatment. Most neuroprotectant showed limited effects in treating ischemic stroke for their short half-lives, potential toxicity, poor specificity of distribution, as well as low targeting efficiency to cross the BBB to achieve effective drug accumulation at the aimed lesions.[52] For instance, the half-life of edaravone *in vivo* is as short as 5.4 min, which seriously hinders the effectiveness of the agent. In addition, biological macromolecules such as superoxide dismutase (SOD) which could efficiently eliminate ROS, and cyclosporine A (CsA) which could inhibit the inflammatory response, have exhibited fine protective effect on damaged neurons. However, these protein or peptide drugs also possess poor stability and short half-lives *in vivo*, and extremely low capability in crossing the BBB[53,54]. Moreover, gene medicines could also be applied for treating stroke, however, it would also encounter the plight of protein or peptide drugs[55,56]. If these neuroprotectant could be successfully delivered to the ischemic brain, these therapeutic molecules are hopeful to play a more effective and lasting protective role.

Currently, in the absence of more effective drugs in treating ischemic stroke, it is necessary to devote pharmaceutical improvements to existing drugs to prolong their half-life *in vivo*, meanwhile, to decrease their non-specific distribution in non-targeting tissues, and to improve their targeting ability to brain lesions to obtain better results. Thus, there is a great demand in developing more efficient and safer delivery strategy of antithrombotic drugs and neuroprotectant for ischemic stroke treatment.

4. Barriers in targeted drug delivery to the damaged brain

The ideal targeted nanomedicine for cerebral ischemia treatment is regarded to be able to deliver drugs to thrombus site or the damaged brain parenchyma effectively, and further to specific cells, such as neurons or glial cells. For therapy with neuroprotectant, although intracranial or intrathecal injection could obtain greater benefits sometimes, their unsustainable drug concentration and invasiveness limits their long-term application, and systemic administration is a more practical method in most cases. Once the nanomedicines enter the blood, they tend to protect the loaded drug from the enzyme degradation; the relatively particle with higher size of nanomedicines could also prevent the drug from being quickly eliminated by the kidney. However,

Table 1 Summary of traditional treatment strategies.

Type of drugs	Therapeutic agent	Mechanism of action	Ref.
Antiplatelet drugs	Aspirin	Selectively inhibits the COX-1 involved in platelet adhesion	40
Anticoagulants	Heparins	Bind to antithrombin to deactivate thrombin and inhibit clotting factors	41
	Warfarin	Vitamin K antagonist, reduces the production of activated clotting factors	41
	Argatroban	Directly Inhibits the thrombin	41
Thrombolytics	Urokinase and alteplase	Activate the plasminogen to form plasmin	42
Neuroprotectant	Memantine	NMDAR antagonist	45
	Nimodipine	Calcium channel blocker	46
	Edaravone	Free radical scavenger	47,48
	Antibody against E-selectin	Limits the infiltration of neutrophils into the ischemic brain	49
	Fingolimod	Reduces the infiltration of lymphocytes into the damaged brain	50

nanomedicines with larger size might be opsonized by plasma proteins and subsequently phagocytosed by the reticuloendothelial system (RES), resulting in non-targeted accumulation in liver and spleen. PEGylation could help the nanomedicines to evade the phagocytosis, achieve longer circulation period in the blood, and reduce its non-specific distribution[57]. PEG would generate hydrophilic sheath on the surface of NPs to shield the opsonization of opsonin and reduce the phagocytosis of macrophages. Longer circulation time is required for nanomedicine delivery, which can increase the chance of nanomedicine of leaking into the brain through the damaged BBB, as well as provide more opportunities for targeting ligands to combine with receptors or transporters. In this section, we will mainly discuss the obstacles and strategies that the neuroprotective nanomedicines encounter before they penetrate into the brain parenchyma across the BBB. Thrombus targeting strategies for thrombolytics-loaded nanomedicine are discussed in Section 5.1.

4.1. BBB

BBB is a special and inherent structure between the brain tissue and blood. It is mainly composed of brain capillary endothelial cells and their tight junctions (TJs), pericytes embedded in capillary basement membrane, and astrocytes end-feet around the capillary (Fig. 2). While restricting the entry of toxic substances into the central nervous system, the BBB also prevents almost all macromolecular drugs and nearly 98% of small molecule drugs from entering the brain tissue. It is difficult for nanomedicines to cross the tight BBB; fortunately, there are strategies that could be adopted to help nanomedicine go cross the BBB and diffuse into the brain parenchyma.

4.2. Strategies for nanomedicines to cross the BBB

4.2.1. BBB disruption and modulation

It is reported that the structure of BBB is partially destroyed during the occurrence of stroke[58,59]. Subsequently, the permeability of BBB to molecules or particles increases, which provides more possibilities for nanomedicine without targeted modification to enter the brain parenchyma. In the early stage, the upregulated expression of caveolin in endothelial cells would enhance the transcellular transport of nanomedicine; the following breakdown of TJs will promote the particles to cross the BBB *via* the paracellular route[59]. There were many unmodified nanomedicines which achieved passive accumulation in brain through the disrupted BBB after stroke[53,55,60–70].

However, the disruption of the BBB is not obvious in the first few hours after the ischemic attack, and this period coincides with the treatment window[58]. Moreover, the crossing of compromised BBB in stroke is often not sufficient for nanomedicines to achieve therapeutically drug concentration. Modifying vehicles with targeting ligand could assist them to cross the intact BBB through receptor-mediated transcytosis as discussed in section 4.2.2.

Recently, some studies adopted BBB modulators to boost the crossing efficiency. The more researched modulators are adenosine 2 A receptor (A2AR) agonist, which bind to the A2AR overexpressed on the luminal and abluminal side of cerebral capillaries during stroke[58,71,72], such as lexiscan, purine nucleotide derivative CGS21680. Activating A2AR could temporally improve the BBB permeability by actively opening the TJs, subsequently helps nanomedicine achieve effective accumulation in the ischemic brain. For instance, edaravone-loaded PEG-PLA micelles were anchored with CGS21680, which acted as both a targeting and signaling molecule, and this strategy simultaneously increases the paracellular transport and receptor-mediated transcellular transport of the nanomedicines[71]. In another study, Lexiscan and an apoptosis inhibitor were co-encapsulated in PEG-PLGA-NPs modified with targeting ligand chlorotoxin. NPs released lexiscan after entering the ischemic site *via* the damaged BBB, which in turn momently improve the BBB permeability to promote the entry of more NPs[58].

4.2.2. Ligand-based targeting

Numerous receptors and transporters are highly expressed on brain capillary endothelial cells to transport glucose, amino acids, proteins, and other nutriments to maintain brain homeostasis[73]. Modifying nanomedicines with aforementioned endogenous ligands or exogenous derivatives could help them cross the BBB actively *via* receptor- or transporter-mediated transcytosis. The studied receptors and transporters mainly include transferrin receptor (TfR), low-density lipoprotein receptor (LDLR) and nicotinic acetylcholine receptor (nAChR). There are also some receptors highly expressed after ischemia and reperfusion, which provides more opportunities for targeted delivery of nanomedicines. In addition to covalently conjugating ligands to nanomedicine, NPs can also achieve active targeting by adsorbing endogenous ligands in the blood. For instance, puerarin-loaded PBCA NPs were coated with polysorbate 80 which could adsorb apolipoprotein E (APOE) in blood. This method enhanced the accumulation of NPs in brain *via* the interaction between APOE and LDLR-related protein (LRP)[74]. The ligands corresponding to the receptors and their applications in targeted delivery by nanomedicines are summarized in Table 2[58,71,74–88].

4.2.3. Biomimetic targeting

Targeted nanomedicines have shown broad applying prospects in treating stroke, though due to the characteristics of exogenous nanomaterials, there is still a risk of being recognized and cleared by the RES. Recently, the bionic nanomedicine delivery strategies based on live cells, cell membrane vesicles and exosomes have been widely researched. Encapsulating nanomedicine onto live

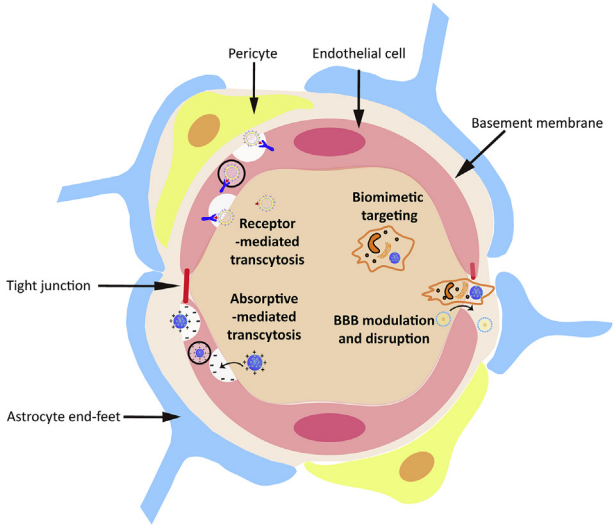


Figure 2 Schematic illustration of the BBB and the strategies for nanomedicines to cross the BBB.

Table 2 Ligands corresponding to receptors and their applications in targeted delivery of nanomedicines.

Receptor	Nanomedicine modified with corresponding ligand	Ref.
SDF-1	PLGA-NPs coated with NSCs membrane that highly expressed CXCR4	75
CXCR4	Thrombin responsive size-shrinkable NPs modified with AMD3100	76
CA 1	Glyburide-loaded betulinic acid NPs	77
TfR and NMDAR	ZL006-loaded liposomes modified with T7 peptide and SHp	78,79
NMDAR	NR_2B_9c-loaded dextran NPs coated with RBCs membrane which modified with SHp	80
Integrin $\alpha v\beta 3$	Curcumin-loaded exosomes modified with cRGD	81
LRP	Puerarin-loaded PBCA NPs coated with polysorbate 80 which could adsorb apolipoprotein E in the blood	74
A2AR	Edaravone-loaded PEG-PLA micelles modified with CGS21680	71
NMDAR1	SOD-loaded nanomedicine modified with anti-NMDAR1 antibody	82
Integrin $\alpha v\beta 3$	PEGylated Ce-NPs conjugated with biotinylated-LXW7	83
LRP	Edaravone-loaded Ce-NPs modified with angiopep-2	84
EPO receptor	AEPO PEGylated liposomes conjugated with AEPO	85,86
MMP-2	Lexiscan-loaded PLGA-NPs modified with targeting ligand chlorotoxin	58
LRP1	NGF-loaded albumin NPs modified with APOE	87
nAChR	miR-124-loaded exosomes modified with rabies virus glycoprotein which could bind the nAChR on BBB	88

cells or coating nanomedicines with endogenous cell membrane as a functional material not only reduces the immunogenicity of nanomedicine, prolongs their circulation time, but also confers an accurate targeting capability on them[89,90].

Neutrophils and macrophages are main targeted cells with inflammatory tropism, as they could respond to chemokine and migrate to damaged brain. The ligand on the cell membrane such as LFA-1 could bind to ICAM-1 highly expressed on capillary endothelial cells, and promote the retention of loaded nanomedicine to enter the brain parenchyma. For instance, targeting ligand PGP-decorated PEG-DGL-CAT NPs hitchhiked the circulating neutrophils in blood *via* the binding between chemoattractant PGP and specific receptor on neutrophils. Neutrophils carrying NPs infiltrated and migrated to the ischemic area where they transfer NPs to damaged neurons through transient intercellular connections[91].

Exosomes are cell membrane vesicles with nanoscale, which can also be used for targeted drug delivery since they retain the membrane protein of the original cells[92,93]. Further targeted modification of cell membrane vesicles and exosomes could improve their targeting ability[60,75,80,81,88]. For instance, cRGD-modified mesenchymal stromal cell (MSC)-derived exosomes loading with curcumin accumulated more at the lesion area, compared to unmodified exosomes, as surface modification improved the active targeting ability of exosomes[81].

4.2.4. *Adsorptive-mediated transcytosis*

Electrostatic interactions between cationic protein/cell penetrating peptide (CPP) with positive charge and brain capillary endothelial cells with negative charge could assist nanomedicine to cross the BBB through adsorptive-mediated transcytosis (AMT). For instance, modification with TAT (belonging to the CPP family) improved the ability of nanoplatelets crossing the BBB to deliver loaded drug[94]. In another research, cationic bovine serum albumin conjugated tanshinone PEG-PLA NPs possessed the ability to attach to the negatively charged brain microvascular lumen sides, and increased the amount of tanshinone in the ischemic brain through AMT[95].

4.2.5. *Bypass the BBB*

In addition to highly invasive intracranial and intrathecal injections, intranasal administration provides a new and feasible route to bypass the BBB. Intranasal administration can mainly deliver nanomedicine to the olfactory bulb or other brain regions through the olfactory nerve. For instance, curcumin-loaded embryonic stem cell exosomes delivered drug into brain intra-nasally, which decreased the level of ROS and infarct volume[96]. In addition, the positively charged vehicle could increase the penetration of nanomedicine into nasal epithelial cells to enhance the transport of drugs to the brain. Arginine-poly-amidoamine ester (e-PAM-R), a biodegradable polycationic ester, was selected to carry anti-HMGB1 siRNA intranasally to improve the delivery efficiency of siRNA[97].

5. Nanomedicines for stroke treatment

5.1. *Nanomedicines improving the efficacy and safety of thrombolytics*

There will be more serious and rapid damage in the ischemic core where the blood flow is lowest within the ischemic regions[98], the cells in ischemic core are doomed to die regardless of any treatments, unless blood supply recovers rapidly. In the ischemic penumbra which is at the edge of the ischemic core, neuronal injury develops slowly due to the existence of microcirculation which is an adjacent capillary network that maintains brain perfusion above the threshold of immediate cell death[13]. Early reperfusion is the main goal of most interventions to ensure cells in penumbra resistant to the ischemia-induced death. However, the effect of thrombolysis with plasminogen activator is limited, for their short half-life and low targeting efficiency. As expected, nanomedicines have been developed to deliver thrombolytics efficiently and precisely *via* physical targeting, ligand-mediated active targeting and biomimetic targeting. Moreover, the well-designed response release further improves the effectiveness and safety of delivering thrombolytics with nanocarriers.

5.1.1. Physical targeting

Physical targeting refers to guiding NPs to the target site with the assistance of external manipulation, or enabling the response release of drug from nanomedicine at the target site under the action of heat or mechanical force such as ultrasound. PEG crossed glycol chitosan soft NPs with good ultrasound response ability were synthesized to deliver urokinase[99,100]. NPs achieved better effect in degrading clots and protecting the BBB, though NPs did not decrease the risk of HT than free protein.

5.1.2. Ligand-mediated active targeting

Modifying vehicle with specific ligand could also confer the targeting ability on thrombolytics. As the fibrin is a major component of thrombus, anti-fibrin antibody-modified polystyrene NPs were applied to deliver alteplase through conjugating proteins on the surface of NPs. Alteplase NPs showed much less activity than free enzyme in the absence of embolus, which would reduce the unspecific cleavage of plasminogen and further decrease the risk of bleeding[101]. Glycoprotein IIb/IIIa (GPIIb/IIIa) receptors on platelet membrane may play a key role in the aggregation of platelets and the formation of thrombus, cyclic Arg-Gly-Asp (cRGD) peptide which could specifically bind the GPIIb/IIIa receptors is employed to mediate targeting thrombolysis, such as cRGD-modified target sensitive streptokinase (SK) liposomes[102–104]. After the interactions between cRGD with activated platelets liposomes consisted of cRGD-palmitic acid underwent destabilization and released encapsulated SK around the clot[102].

Apart from single-targeted ligand modification, there are some studies on dual ligand-modified nanomedicine with higher targeting efficiency[105]. The overexpressed P-selectin on activated platelets mediate the interaction between thrombocytes. Inspired from this mechanism, dual-targeted nanovesicles modified with cRGD and P-selectin targeting peptide were developed to deliver SK[105]. Nanovesicles composed of glycerophospholipids achieved stimuli-responsive release of drugs after locating at the target site, as glycerophospholipids could be cleaved by phospholipase A2 which is overproduced by activated platelets and leukocytes in thrombus[105] (Fig. 3). Nanovesicles showed better therapeutic effects, and the risk of bleeding was reduced compared to free drug.

5.1.3. Biomimetic targeting

Considering the role of platelets in thrombus formation, bioengineered nanoplatelets were developed for treating ischemic stroke. The polymeric NP core was coated with platelets membrane, the membrane was conjugated with TAT-peptide-coupled alteplase linked with amino acid sequences which could be cleaved by thrombin[94]. The nanoplatelets can avoid the recognition and phagocytosis of the RES, and adhere to the thrombus site to prolong the retention of NPs and achieve responsive release of alteplase. Biomimetic targeting nanomedicines based on cell membrane have become an emerging effective strategy in treating ischemic stroke.

In conclusion, targeted delivery of plasminogen activator *via* nanomedicine could increase the accumulation of drugs at the thrombus site, thereby reducing the dosage and minimizing the side effects of thrombolytics. The strategies, including

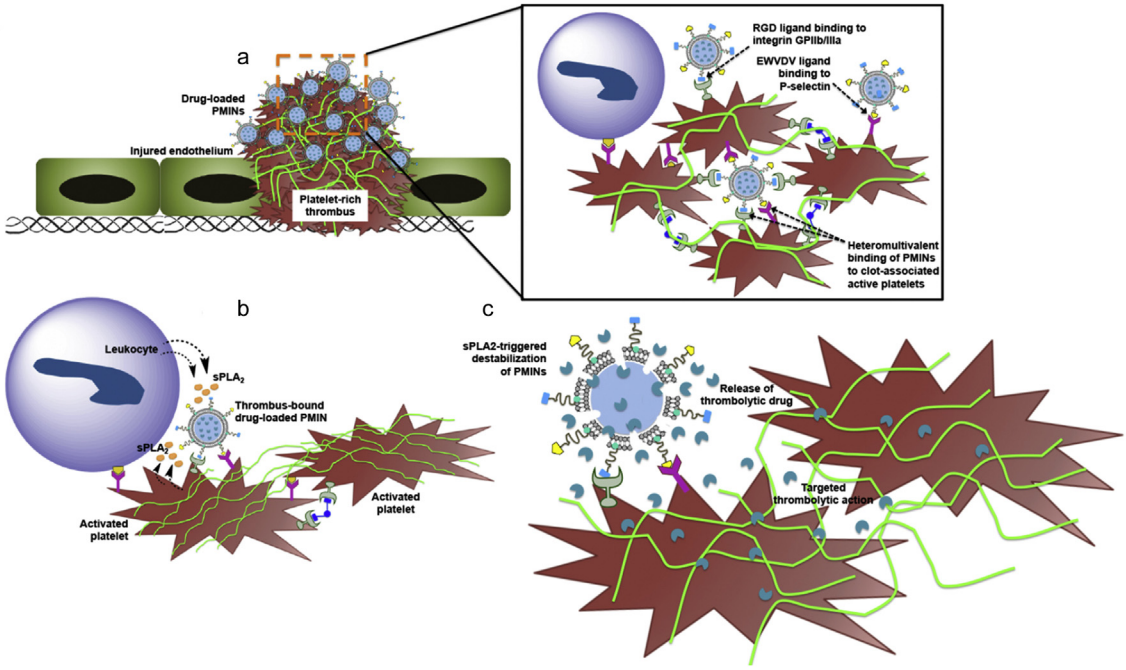


Figure 3 Schematic diagram of targeted thrombolysis using nanovesicles inspired from activated platelets. (a) Nanovesicles bind the activated platelets in thrombus through the interaction between RGD and GPIIb-IIIa, as well as P-selectin targeting peptide and P-selectin. (b) Nanovesicles composed of glycerophospholipids achieve responsive release of drugs after locating at the target site, as the glycerophospholipids are cleaved by phospholipase A2 overproduced by activated platelets and leukocytes in thrombus. (c) Drug released from degraded nanovesicle realizes targeted fibrinolysis. PMINs: platelet microparticle-inspired nanovesicles. Reprinted with the permission from Ref. 105. Copyright © 2017, Elsevier Ltd.

Table 3 Summary of targeted delivery of thrombolytics with nanomedicines.

Strategy	Target	Ligand	Nanomedicine	Achievement	Limitation	Ref.
Physical targeting			Urokinase-loaded NPs	Enhanced clots degradation and BBB protection	No reduction in the risk of HT	99,100
Ligand-mediated active targeting	Fibrin	Anti-fibrin antibody	Alteplase-loaded NPs	Showed less activity in the absence of embolus	Lack of experimental data *in vivo*	101
	GPIIb/IIIa receptors	cRGD peptide	Target sensitive SK-loaded liposomes	Target sensitive release of SK; enhanced clot dissolution *in vivo*	No data about the risk of HT	102−104
	GPIIb/IIIa receptors P-selectin	cRGD peptide P-selectin targeting peptide	Phospholipase A2 responsive SK-loaded nanovesicles	Reduced the risk of bleeding	No data about the risk of cerebral hemorrhage	105
Biomimetic targeting	Thrombus	Membrane proteins on platelets	Alteplase-loaded NPs coated with platelets membrane	Achieved responsive response of alteplase in thrombus	No data about the risk of cerebral hemorrhage	94

achievement and limitation in targeted delivery of thrombolytics are summarized in Table 3[94,99−105].

5.2. *Nanomedicines delivering oxygen to the ischemic brain*

For patients who are not suitable for thrombolysis or have shown microcirculation impairment due to the formation of microthrombus, delivering sufficient O_2 to the ischemic penumbra is a potential method. Hemoglobin (Hb) is a natural O_2 carrier, but short half-life and hypertension response limit its clinical application[106,107]. Liposomal Hb (LHb) could not only prolong the circulation time of Hb *in vivo*, but also deliver sufficient O_2 to cross the obstacles in the microcirculation and get to the penumbra where red blood cells (RBCs) can seldom reach[108,109]. In a rat thrombosis model induced by photochemistry, fluorescein-labeled RBCs were mainly observed in larger vessels, whereas fluorescent liposomes filled all vessels including capillaries in a manner similar to plasma[108]. LHb significantly reduced the infarct volume of ischemic penumbra, compared to free Hb and homologous blood which contains lots of RBCs carrying Hb[109]. Moreover, LHb can also significantly attenuate the infarction size in transient ischemic model when infused immediately after the middle cerebral artery occlusion (MCAO)[110,111]. Though delivering O_2 could rescue dying neurons by relieving hypoxia in the penumbra, too much O_2 after reperfusion may cause the significant increase in ROS, the timing and dosage of administration need further investigation.

5.3. *Nanomedicines regulating ion imbalance and excitotoxicity*

Ion imbalance and excitotoxicity occur upstream during the ischemic cascade, and regulating the progression of these events is expected to inhibit the amplification of damage in the early stage of stroke as well as produce more effective intervention outcomes. The sulfonylurea receptor 1-transient receptor potential melastatin-4 (SUR1−TRPM4) complex is a nonselective cation channel which is sensitive to ATP, and the expression of the channel is upregulated in ischemic neurons, astrocytes and capillaries[112]. Depletion of ATP in the ischemic brain leads to the continued opening of SUR1-TRPM4 channel which increases the influx of Na^+, causes the depolarization of cells and cytotoxic edema, and finally leads to the necrocytosiss and brain swelling[113]. Glyburide is an inhibitor of SUR1−TRPM4 complex, and has shown treating effect in reducing edema and infract volume in animal and human[114]. However, glyburide is a substrate for P-glycoprotein, which limits its ability in penetrating the BBB to realize the effective therapeutic concentration[115]. As expected, several nanomedicines have been developed to deliver glyburide, such as poly (L-lactide-*co*-glycolide) nanoparticles (PLGA-NPs)[75], thrombin-responsive size-shrinkable brain targeting NPs[76], glyburide-loaded betulinic acid NPs[77]. It has been reported that neural stem cells (NSCs) could migrate to the injured site due to the interaction between C-X-C chemokine receptor type 4 (CXCR-4) and ligand stromal cell-derived factor-1 (SDF-1) which is overexpressed in the ischemic brain. PLGA-NPs coated with membrane derived from NSCs overexpressed CXCR4 demonstrated higher effects in enhancing brain targeting delivery of glyburide, which significantly reduced the infract volume[75] (Fig. 4). Thrombin-responsive NPs could shrink their size after crossing the damaged BBB and arriving at the ischemic site where thrombin is highly expressed, which enhanced the penetration of NPs in the brain parenchyma[76]. Betulinic acid NPs realized penetrating the ischemic brain through the interaction between betulinic acid and cannabinoid receptor one overexpressed on the damaged BBB, which can improve the delivery efficacy of glyburide to the brain[77].

Postsynaptic density protein-95 (PSD-95) participates in the activation of neuron nitric oxide synthase (nNOS) mediated by NMDAR *via* the formation of NMDAR/PSD-95/nNOS complex, disrupting the association between nNOS and PSD-95 provides a method to ameliorate the damage caused by excitotoxicity[116]. ZL006 could block the nNOS-PSD-95 interaction selectively, however, the drug has obvious drawbacks in the ability to accumulate in the ischemic brain[78]. Dual targeted liposomes conjugated with T7 peptide and stroke-homing peptide (SHp) were developed to deliver ZL006 accurately, and T7 peptide could help the liposomes transport across the BBB through TfR-mediated

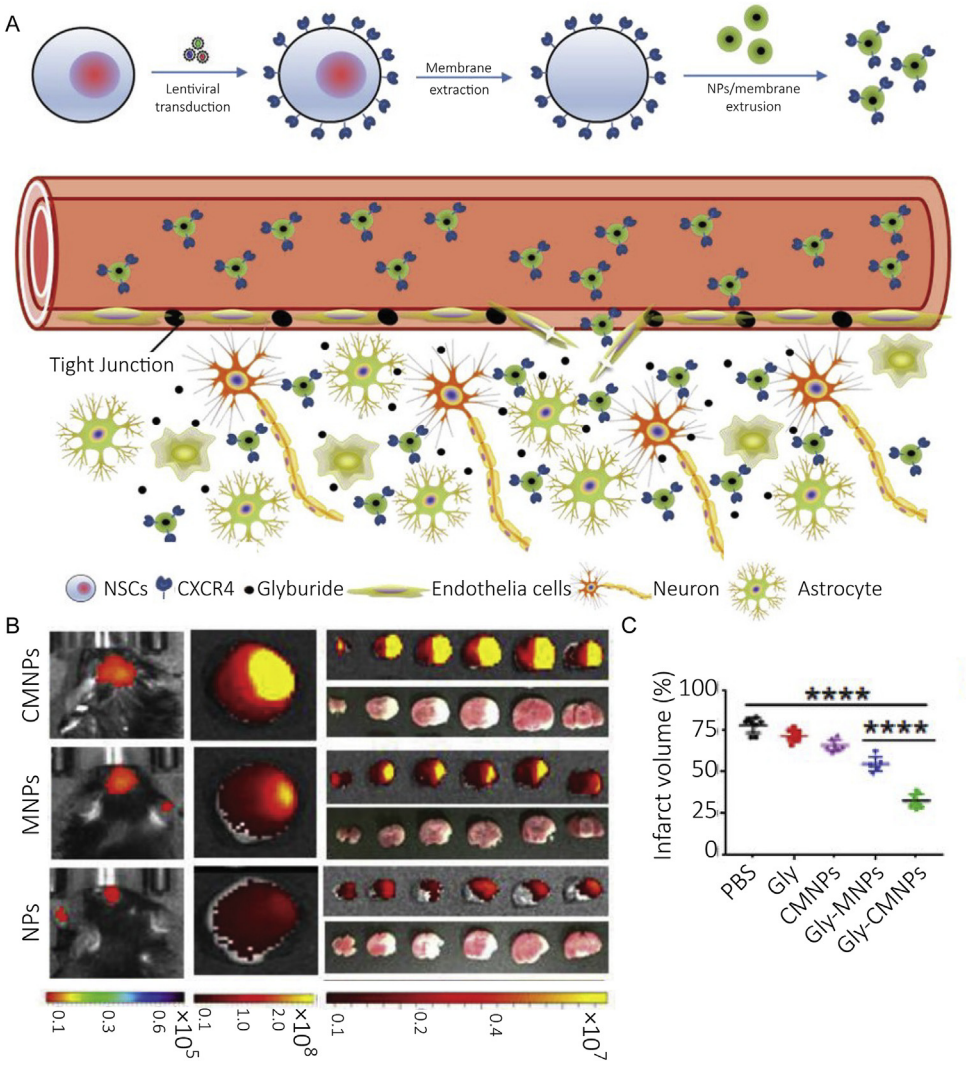


Figure 4 (A) Scheme of targeted delivery of PLGA NPs coated with membrane derived from NSCs which overexpressed CXCR4 to the ischemic brain. Lentiviral transduction was applied to transform NSCs to overexpress CXCR4. PLGA NPs accumulate in the ischemic area after intravenous injection, and glyburide entrapped in NPs are released to rescue neurons. (B) The accumulation of IR-780-loaded NPs in ischemic brain ($n = 3$), CMNPs showed significantly higher accumulation than MNPs or NPs. (C) Infarct volume ($n = 5$), of MCAO mice treated with different formulations. CMNPs: CXCR4-overexpressing membrane-coated NPs; Gly: glyburide; MNPs: membrane-coated NPs. Reprinted with the permission from Ref. 75. Copyright © 2019, WILEY-VCH.

transcytosis[24,117], SHp preferentially homed to the ischemic territory and mediated the endocytosis of liposomes by damaged neurons *via* glutamate receptors[79]. Dual targeted liposome could significantly reduce the infarct volume, and improve neurological outcomes in stroke rats, compared with T7 peptide or SHp-modified liposomes. In another research, dextran NPs coated with RBCs membrane modified with SHp were developed to deliver NR2B9c peptide which could prevent the binding of PSD-95 to NMDAR to reduce the excitotoxicity induced by glutamate[80].

Tacrolimus could inhibit the activation of Ca^{2+}-dependent calcineurin through binding the tacrolimus-binding protein in neurons. However, frequent use of high-dose tacrolimus to achieve good therapeutic effect has shown side effects[118]. Liposomal tacrolimus can improve the solubility of drug, and reduce the administration dosage without affecting the neuroprotective efficacy, as liposomal drug diffused more into the ischemic brain than free drugs through the damaged BBB[60]. Table 4 summarizes the strategies in targeted delivery of nanomedicines which could deliver oxygen, regulate ion imbalance and excitotoxicity.

5.4. *Nanomedicines reducing oxidative stress*

Therapy employing antioxidants to scavenge excessive ROS, not only attenuates the oxidative stress injury, but also reduces downstream apoptosis and inflammatory responses. Therefore, antioxidant therapy now is a major method to treat injury caused by the ischemia and reperfusion. Antioxidants in preclinical research mainly include small molecule compounds, antioxidase

Table 4 The strategies, including achievements and limitations in targeted delivery of nanomedicines which could deliver oxygen, regulate ion imbalance and excitotoxicity.

Phase of cascade	Target	Nanomedicine and intervention strategy	Achievement	Limitation	Ref.
Oxygen deficiency	Passively	Hb-loaded liposomes which carry oxygen	Delivered sufficient O_2 to the penumbra where Hb-containing RBCs rarely reach	Too much O_2 after reperfusion might cause the significant increase of ROS	108−111
Ion imbalance					
SUR1-TRPM4 complex	SDF-1	PLGA-NPs coated with NSCs membrane that highly expressed CXCR4	Reduced the infract volume	Long term administration might increase the risk of hypoglycemia	75
Inhibitor: glyburide	CXCR4	Thrombin responsive size-shrinkable NPs modified with AMD3100	Enhanced the penetration of NPs in the brain		76
	CA 1	Glyburide-loaded betulinic acid NPs	Reduced the oxidative stress		77
Excitotoxicity					
NMDAR/PSD-95/nNOS complex	TfR and NMDAR	ZL006-loaded liposomes modified with T7 peptide and SHp	Reduced the infarct volume and improved neurological outcomes		78,79
	NMDAR	NR_2B_9c-loaded dextran NPs coated with RBCs membrane modified with SHp	Ameliorated neurological deficit		80
Ca2+-dependent calcineurin	Passively	Tacrolimus-loaded liposomes	Suppressed the expansion of brain damage without side effects	NPs mainly presented in the ischemic core rather penumbra	60,118

CA 1, cannabinoid receptor 1.

and inorganics. Nanomedicines could improve the pharmacokinetic performance of antioxidants, and assist them to cross the BBB to accumulate at the damaged brain.

Many small molecule antioxidants are plant-derived phenolic and keto compounds, which could react with ROS, such as baicalin[119], luteolin[120], curcumin[121], resveratrol[61], tanshinone[95], and puerarin[74]. However, the poor solubility, short half-livest and limited BBB penetrability restrict their application. Nanotechnology-based delivery strategies have been developed to solve these problems, such as resveratrol albumin NPs[61], cationic BSA conjugated tanshinone PEG-PLA NPs[95], curcumin-loaded NPs[62,121,122], curcumin-loaded exosomes[96], baicalin-loaded liposome[63], luteolin-loaded PEG-PLGA micelles[64,123], and puerarin-loaded poly-butylcyanoacrylate (PBCA) NPs[74]. Taken curcumin as an example, hydrophobic curcumin is easy to be metabolized and eliminated *in vivo*, and it is also poorly absorbed by cells. PLGA-NPs-containing curcumin showed higher uptake in neurons than free drug, as cells internalized NPs through endocytosis rather than passive diffusion, and NPs exerted more efficient antioxidative activity on neurons treated with H_2O_2[62,121,122]. In addition, curcumin entrapped in PEG-PLA NPs realized higher accumulation in the ischemic penumbra through the damaged BBB, compared with free curcumin[62]. The NPs can extend the half-life of curcumin *in vivo*, which increased the chance of the drug penetrating into the damaged brain during the opening window of BBB. In another study, curcumin-loaded embryonic stem cell exosomes not only improved the stability and solubility of drug, but also solved the problem of poor absorption. Moreover, exosomes, which possess the ability to bypass the BBB upon administrated intra-nasally, could transport more drugs into the ischemic brain. Therefore, treatment with embryonic stem cell exosomes loaded with curcumin can significantly decrease the ROS level in the damaged brain, reduce the area of infarct, and attenuate the breakdown of neurovascular units[96].

In addition to natural antioxidants derived from plant, there are also many synthetic small molecule antioxidants, such as edaravone, nitroxyl radicals 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). PEG-PLA micelles modified with CGS21680 were developed to prolong the circulation time of edaravone[71], and the BBB modulation strategy could help the edaravone micelles realize more effective accumulation in the ischemic brain and rescue more neurons. As a nitroxyl radical, TEMPO is found to be quickly reduced by antioxidase *in vivo*, and administration with TEMPO might induce hypotension[65]. pH-Sensitive PEG-poly-chloromethylstyrene (PEG-*b*-PCMS) micelles were designed to prolong the half-life of TEMPO, as well as to reduce the dosage and toxicity[124]. The chloromethyl groups of PEG-*b*-PCMS were converted to TEMPOs *via* the amination with 4-amino-TEMPO, and the micelles would decompose and expose the TEMPO upon the protonation of amino groups in intracellular acidic compartments[124,125]. TEMPO-contained micelles significantly reduced the production of ROS in the ischemic area, and limited the BBB damage[66].

In some cases, the polymers that construct the nanomedicines could also scavenge the ROS and preserve the damaged neurons, such as ROS-sensitive phenylboronic ester-contained polymers in micelles and NPs[23,80], nanomedicines would collapse and simultaneously release the encapsulated drug when eliminating the

high-level ROS. In an interesting study, melanin, a heterogeneous biological polymer, itself can form highly water-dispersible NPs which have the capability against multiple ROS[67]. Coincidentally, betulinic acid NPs also exhibited antioxidant capacity to reduce the oxidative stress and infarct volume in a dose-dependent manner[77].

SOD can convert superoxide anion to less toxic H_2O_2, catalase (CAT) can convert H_2O_2 to H_2O and O_2, and MCAO mice that overexpresses SOD or CAT showed less injury after reperfusion[126,127]. However, the short half-life and low BBB penetrability greatly limit the application of exogenous antioxidase for stroke treatment[54,128]. Liposomes, PBCA NPs, and PLGA-NPs extended the half-life of SOD, and the biphase opening of the BBB after reperfusion helped them achieve enhanced accumulation of SOD in the ischemic brain[82]. Modification with anti-NMDAR1 antibody helped liposomes and NPs realize enhanced uptake by neurons, which showed significant improvement in reducing the level of oxidative stress and infract volume, compared to non-targeted nanomedicines and free SOD[82]. In addition, rats receiving PLGA-NPs loaded with CAT and SOD immediately after reperfusion showed less infiltrated neutrophils and caspase-3 positive cells in the brain than animals receiving alteplase alone, as reducing oxidative stress is beneficial to inhibit the downstream cascade reaction[68]. Moreover, targeting inflammatory cells circulating in blood mentioned above provides new ideas for targeted delivery of antioxidase[91].

Ceria nanoparticles (Ce-NPs) which possess the ability to scavenge multiple ROS were applied to treat ischemic stroke[69]. Though the size of Ce-NPs was about 20 nm, the concentration of ceria in non-ischemic brain was low. However, there was significant improvement in the concentration of Ce-NPs in the ischemic brain, as the BBB was damaged after ischemia. In order to realize better treatment, PEGylated Ce-NPs was conjugated with biotinylated-LXW7, targeted modified Ce-NPs could cross the BBB through the interactions between LXW7 and $\alpha v\beta 3$ which is upregulated on BBB after stroke, Ce-NPs modified with LXW7 exhibited better effects in reducing infarction size and the degree of BBB breakdown than Ce-NPs[83]. In another study, PEGylated Ce-NPs modified with angiopep-2 were used to load edaravone through van der Waals forces, modified Ce-NPs accumulated in the brain tissue *via* the LRP1-mediated transcytosis. Edaravone-loaded Ce-NPs exerted synergistic antioxidant effect to rescue damaged neurons, and protected BBB from further damage[84]. Inorganic NPs which have good uniformity are easy to prepare and modify, and have been gradually applied in the field of treatment and drug delivery, though their biosafety still needs further serious investigation. Table 5 summarizes the strategies in targeted delivery of nanomedicines in reducing oxidative stress.

5.5. *Nanomedicine reducing apoptosis*

Apoptosis is an intermediate event of the ischemic cascade. Reducing apoptosis could not only rescue the stressed neurons, but also weaken the downstream inflammation and immune response, which is conducive to inhibit the deterioration of stroke. Most of the antioxidants mentioned above also have anti-apoptotic ability, as oxidative stress is a main cause of apoptosis[61,62,66,68,81,82]. In addition, the more researched anti-apoptotic drugs are mainly biomacromolecules, and ligand-modified NPs and exosomes have been developed to deliver these drugs[58,85,86,129]. Asialoerythropoietin (AEPO) could bind the erythropoietin receptor expressed on neurons, and protect the neurons from apoptosis *via* the activation of protein kinase Akt-1/protein kinase B and extracellular signal-regulated kinases[130,131]. PEGylated liposomes could prolong the circulation period of AEPO, and the interactions between AEPO and erythropoietin receptor conferred the neuron targeting ability of liposomes upon crossing the damaged BBB. There was more accumulation and retention of ^{125}I-labeled AEPO-liposome in the ischemic hemisphere, in rats which were injected with free AEPO or liposomal AEPO. A single injection of AEPO-liposomes significantly inhibited neuronal apoptosis and improved neurological outcomes, compared to administration of free AEPO[85,86]. Moreover, anti-apoptosis protein BCL-2 could prevent the caspase apoptosis cascade, pigment epithelium-derived factor is believed to protect neurons from apoptosis through activation of ERK1/2 and induction of BCL-2[132]. For instance, exosomes origin from adipose-derived mesenchymal stem cells which can overexpress pigment epithelium-derived factor could reduce brain damage by inhibiting neuronal apoptosis[129]. In another study, Nogo-66 receptor antagonist Nogo extracellular peptide 1−40 could reduce the increased caspase-3 activity in ischemic brain[133], and the peptide encapsulated in PLGA-NPs modified with targeting ligand chlorotoxin significantly reduced the infarct volume and enhanced the survival of ischemic mice, compared with free drug[58]. Table 6 summarizes the strategies in targeted delivery of nanomedicines in reducing apoptosis.

5.6. *Nanomedicines regulating inflammation and immune response*

In the ischemic brain, injured cells can release DAMPs which would activate the innate immunity and initiate the process of inflammatory response[134]. For instance, HMGB1 activates pattern recognition receptors on microglia, which will upregulate the expression of pro-inflammatory gene through multiple signaling pathways, such as the transcription factor nuclear factor-κB (NF-κB)[29,30]. Numerous inflammatory mediators are NF-κB-dependent, such as ICAM-1, IL-2,6, TNF-α and chemokines. NF-κB could also be activated by ROS or cytokines like TNF-α, IL-2, and IL-6[30]. In addition, pattern recognition receptors are also found in the cerebral endothelium, astrocytes and neurons[29]. The inflammatory mediators released from the brain cells might spread to the blood vessels to amplify the expression of inflammatory mediators, which drives the infiltration of leukocytes into the ischemic brain and aggravate the progress of inflammatory response[13,31−37]. The inflammatory and immune responses would dramatically expand the brain damage, and curbing the occurrence and progression of inflammation is particularly important for protecting the damaged brain. In addition to inhibiting the upstream events of the ischemic cascade, inhibiting vital components in the inflammatory response, such as pro-inflammatory mediators and cells, could also exert anti-inflammatory effects.

5.6.1. *Nanomedicines inhibiting pro-inflammatory mediators*

As HMGB1 plays an important role in inflammatory response, inhibiting the production of HMGB1 might be an effective way to reduce the injury caused by inflammation[29,30]. Gene silencing mediated by RNA interference technology could regulate the specific protein functions, yet the delivery of small interfering

Table 5 The strategies, including achievements and limitations in targeted delivery of nanomedicines in reducing oxidative stress.

Phase of cascade	Target	Nanomedicine and intervention strategy	Achievement	Limitation	Ref.
Oxidative stress					
Small molecule antioxidants	Passively	Curcumin-loaded PLGA NPs	Prevented neurons from oxidative damage	No experimental data *in vivo*	121,122
	Passively	Curcumin-loaded PEG-PLA NPs	Protected the BBB against oxidative stress; ameliorated neuronal apoptosis and the release of inflammatory mediators.	Conjugating with targeting ligand might further improve the treatment effect	62
	Bypass the BBB	Curcumin-loaded embryonic stem cell exosomes	Delivered drug into brain intranasally, decreased the level of ROS	The safety of nasal administration has not been investigated	96
	Integrin $\alpha v\beta 3$	Curcumin-loaded exosomes modified with cRGD	Downregulated activated microglia and the expression of inflammatory cytokines		81
	Passively	Resveratrol-loaded albumin NPs	Reduced oxidative stress and the apoptosis of neurons	Passive targeting might not be suitable for long-term administration	61
	AMT	Tanshinone-loaded PEG-PLA NPs conjugated with cationic BSA	Inhibited the inflammatory cascades	There is risks of being phagocytized by the RES	95
	Passively	Baicalin-loaded liposomes	Improved the accumulation of drug in the ischemic brain	Non-PEGylated liposomes were mainly phagocytized by the RES	63
	Passively	Luteolin-loaded PEG-PLGA micelles	Inhibited oxidative stress and decreased the release of inflammatory mediators	Targeting efficiency might need further improvement	64
	LRP	Puerarin-loaded PBCA NPs coated with polysorbate 80	Reduced the infarct volume		74
	A2AR BBB modulation	Edaravone-loaded PEG-PLA micelles modified with CGS21680	Exerted a more persistent ROS eliminating effect and rescued more neurons	The safety of BBB modulator needs further study	71
	Passively	PEG-*b*-PCMS-TEMPO micelles	Suppressed oxidative stress and apoptosis, protected neurovascular units	The study did not reveal the effect and toxicity of NPs in subacute phase	65,66
	Passively	Melanin-loaded NPs conjugated with PEG	Showed antioxidative effects against multiple RONS, suppressed the expression of inflammatory cytokines	The potential toxicity of the degradation byproducts of PEG-MeNPs needs further investigation	67
Antioxidase	NMDAR1	SOD-loaded liposomes, PBCA NPs, and PLGA-NPs; nanomedicines were modified with anti-NMDAR1 antibody	Reduced the oxidative stress and apoptosis, and inhibited the release of inflammatory mediators	The effect of administration in subacute phase is not satisfactory	82
	Passively	CAT/SOD-loaded PLGA-NPs	Mitigated apoptosis and inflammatory response; promoted neurogenesis	Carotid injection is not conducive to long-term administration	68
	Biomimetic targeting	PEG-DGL-CAT NPs decorated with PGP, which could hitchhike the circulating neutrophils	Reduced the infarct volume *via* anti-oxidative effect	The fate of NPs in neutrophils needs further investigation	91
Inorganics	Passively	PEGylated ceria NPs	Scavenged ROS and reduced apoptosis	Targeting ability need further improvement	69
	Integrin $\alpha v\beta 3$	PEGylated Ce-NPs conjugated with biotinylated-LXW7	Showed better effects in mitigating the oxidative stress and apoptosis	There was no improvement in neurological function	83
	LRP	Edaravone-loaded Ce-NPs modified with angiopep-2	Exerted synergistic ROS scavenging ability		84

A2AR, adenosine 2 A receptor; BSA, bovine serum albumin; LRP, lipoprotein receptor-related protein; RONS, reactive oxygen and nitrogen species.

Table 6 The strategies, including achievements and limitations in targeted delivery of nanomedicines in reducing apoptosis.

Phase of cascade	Target	Nanomedicine and intervention strategy	Achievement	Limitation	Ref.
Apoptosis	EPO receptor	AEPO conjugated PEGylated liposomes	Achieved strengthened accumulation in neurons, inhibited neuronal apoptosis	It might increase the risk of hematopoietic effect	85,86
	MMP-2 and BBB modulation	Nogo extracellular peptide encapsulated in lexiscan-loaded PLGA-NPs modified with targeting ligand chlorotoxin	Reduced the infarct volume and enhanced the survival of ischemic mice	The safety of lexiscan needs further investigation	58
	Biomimetic targeting	Exosomes derived from ADSCs that overexpress PEDF	Ameliorated neuronal apoptosis		129

PEDF, pigment epithelium-derived factor.

RNA (siRNA) *in vivo* limits the therapeutic application of this technology. Arginine-poly-amidoamine ester (e-PAM-R) was chosen as the carrier to improve the delivery and transfection efficiency of anti-HMGB1 siRNA[56]. The transfection efficiency of e-PAM-R/siRNA complex was thus improved, as reverse transcription-polymerase chain reaction revealed that there was significant downregulation of HMGB1 mRNA levels in primary cortical cultures treated with NMDA or H_2O_2, and treatment with e-PAM-R/siRNA reduced the neuronal death. E-PAM-R can successfully deliver siRNA into the brain cells, since there was notable reduction in HMGB1 level and infarct volume after intranasal delivery of e-PAM-R/siRNA in stroke rats[135].

In addition to HMGB1, TNF-α is also an important inflammatory mediator. CsA is a hydrophobic peptide consisting of 11 amino acid residues, which could inhibit the release of TNF-α and IL-1, and exert anti-inflammatory and neuroprotective activities. However, CsA is effective only at high-dose administration, which results in severe side effects such as seizure. Liposomal CsA showed significant suppression of inflammation and reduction of infarct size, and animals receiving liposomal CsA did not show obvious side effects[53].

As one part of the innate immunity, complement system is also activated in the ischemic brain. After the ischemia and reperfusion, injured neurons expressed complement component C1q, C3 and C5, microglia and astrocytes can also produce C1q and C3[10–12]. C3 induces the phagocytosis of neurons by microglia through the interactions between C3b deposited on the neuronal surface and complement receptor three on microglia[12]. Mice deficient in C3 were protected from reperfusion injury, which showed that inhibiting complement activation is a potential treatment method for stroke[11]. To improve the efficiency of RNA interference, PEG-PLA NPs were applied to carry anti-complement C3 siRNA (C3-siRNA) with the assistance of cationic lipid, which is believed based on the electrostatic interactions. NPs that can deliver siRNA into the damaged brain and subsequently engulf by the microglia, and reduced the level of C3 in microglia and microglial neurotoxicity. Moreover, gene therapy with C3-siRNA-PEG-PLA-NPs decreased the infiltration of inflammatory cells and the level of pro-inflammatory mediators, and improved neurological outcomes[55].

5.6.2. *Nanomedicines regulating inflammatory cells*

As the resident immune cells in the brain, microglia is rapidly activated and then migrate to the damaged site under the action of DAMPs and chemokines[136]. Although activated microglia are generally believed to exacerbate the damage, some studies have shown that unlike the classically activated M1-phenotype microglia with pro-inflammatory activity, alternatively activated M2-phenotype microglia could exert anti-inflammatory effects[137]. In the peri-infarct region, newly recruited microglia are mainly M2-phenotype, but M1-phenotype gradually occupy main position to cause brain damage[138]. Therefore, promoting the microglial polarization from M1 to M2 in the acute phase of stroke might be beneficial to reduce the inflammatory response at the lesion site.

The main pro-inflammatory cytokines secreted by M1 microglia are NF-κB-dependent, such as IL-1, IL-6, TNF-α, and regulating the NF-κB signaling pathway may promote the phenotypic transition from M1 to M2. Since NF-κB could also be activated by ROS, eliminating ROS might help achieve the phenotypic shift[62,95]. For instance, PEGylated Ce-NPs can drive microglial polarization from M1 to M2 *via* scavenging multiple ROS, which is in favor of the resolution of inflammation and reduction of cell death[139].

Nanomedicines have also been used to deliver small molecules to promote the phenotypic transition of microglia. Rapamycin could promote the microglia polarization from M1 to M2, yet the poor BBB permeability limits its application. Fibrin-binding peptide-modified micelles that contain ROS-sensitive phenylboronic ester units were applied to deliver rapamycin into the damaged brain. The modified micelles could realize higher retention in the thrombus site and subsequently accumulated in the ischemic brain. Micelles would collapse and release entrapped rapamycin response to the high-level ROS in the ischemic brain, which could protect neurons through eliminating ROS, and the liberated rapamycin would promote the phenotypic transition of microglia from M1 to M2, thereby inhibiting the damage of neurons and reducing the inflammation response[23] (Fig. 5). The double-sided role of microglia reveals that the treatment of stroke should shift from simply inhibiting the activity of microglia to regulating the balance of cell phenotype.

5.6.3. *Nanomedicines for pretreatment with TNF-α*

Previous research has shown that patients with transient ischemic attack (TIA) preceding ischemic stroke had better neurological outcome, and this ischemic tolerance was related to the overexpression of TNF-α induced by TIA[135]. Although TNF is generally considered to be a proinflammatory factor, pretreatment

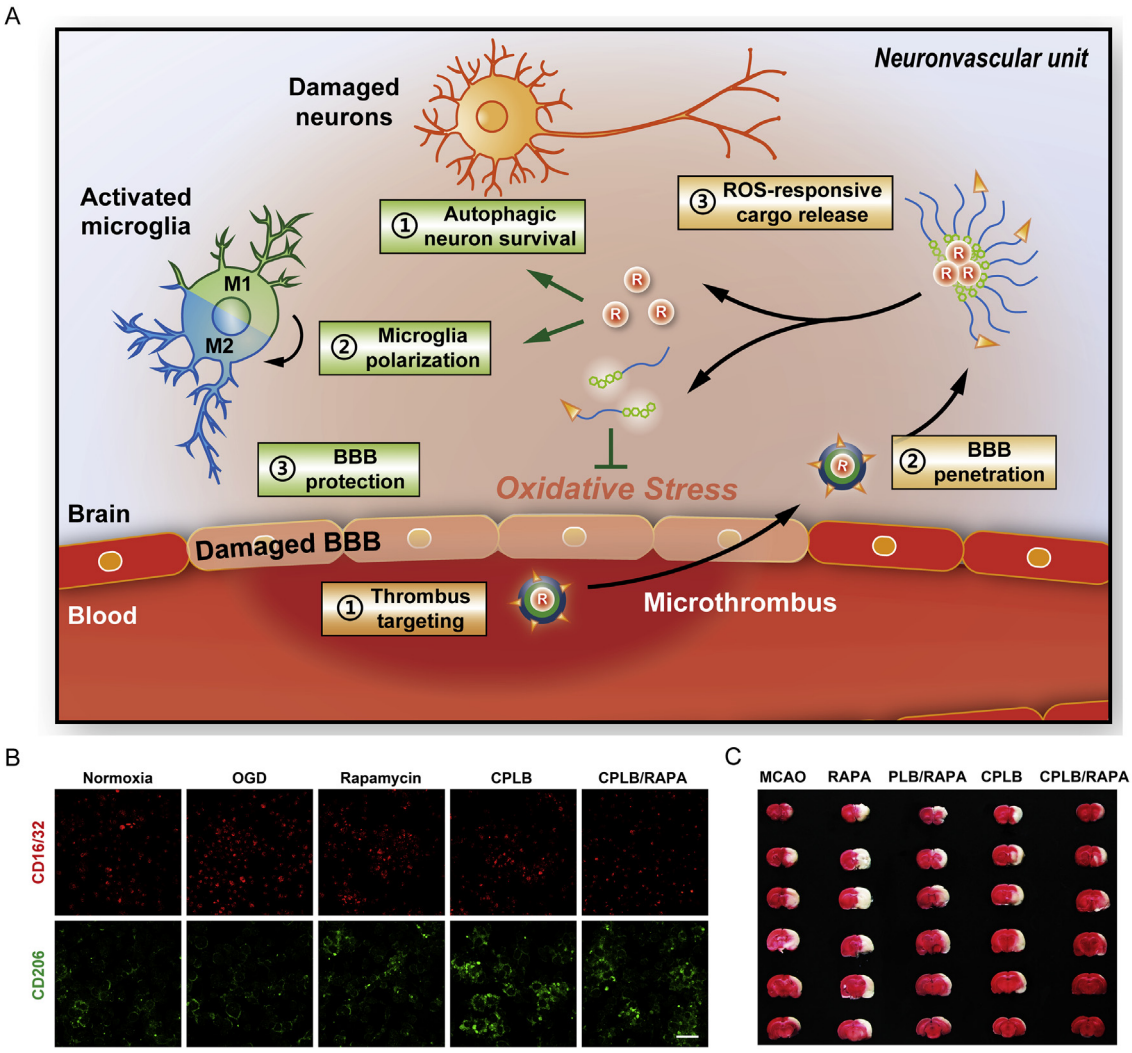


Figure 5 (A) Schematic diagram of CPLB/RAPA micelle in modulating the damaged brain suffered from ischemia: 1) micelle bind the microthrombus through the interaction between fibrin and fibrin-binding peptide; 2) micelle accumulate in the ischemic brain *via* crossing the damaged BBB; 3) ROS initiate the release of rapamycin, rapamycin promote the phenotypic transition of microglia from M1 to M2, thereby inhibiting the damage of neurons and reducing the inflammation response. (B) Immunostaining of M1 (red) and M2 (green) microglia treatment with different formulations (scale bar = 50 μm). (C) Representative TTC staining images of ischemic brain from rats with different treatment. RAPA: rapamycin; PLB: PEG-phenylboronic ester modified polylysine; CPLB: fibrin-binding peptide CREKA modified PLB. Reprinted with the permission from Ref. 23. Copyright © 2019, WILEY-VCH.

with TNF-α could also induce the production of anti-inflammatory mediators such as manganous superoxide dismutase through activating the NF-κB signaling pathway[140–142]. Pretreatment with TNF-α significantly reduced the brain injury caused by following ischemia in mice, yet the half-life of TNF-α is short, which limit its application[143]. PEG-*b*-(poly (ethylenediamine L-glutamate)-*g*-poly (L-lysine)) was applied to load negatively charged TNF-α *via* the electrostatic interactions, and the circulation time of TNF-α *in vivo* was prolonged. Preconditioned with TNF-α-loaded NPs to induce the ischemic tolerance would attenuate the oxidative stress damage and the inflammatory activity, and reduce the neuronal apoptosis level in the ischemic brain after reperfusion[70]. Pretreating to induce ischemic tolerance provides a new idea for the treatment of ischemic stroke, as for patients who have experienced TIA, pretreatment with effective nanomedicine might reduce the severity of following stroke.

5.7. *Nanomedicines promoting tissue repair*

Post-ischemic inflammation would subside gradually, and the damaged brain will undergo a structural and functional recovery process to return to homeostasis. Emerging evidences have shown that the resolution of inflammation is an active process benefiting from the action of anti-inflammatory mediators and immune cells[144]. This process comprises the elimination of dead cells, the establishment of an anti-inflammatory microenvironment, and the production of growth factors (GFs) that promote the repair of brain[13]. Resident microglia and infiltrated macrophages are main phagocytes responsible for the clearing of dead cells after stroke[145,146]. TGF-β and IL-10, two of the most important anti-inflammatory mediators, which are secreted in concert with the phagocytosis of dead cells, play a vital role in the establishment of anti-inflammatory microenvironment which is in favor of tissue

repair[147]. As M2 microglia are responsible for the phagocytosis to remove cell debris and the secretion of anti-inflammatory factors to restrict brain damage, the nanomedicines that regulate the microglial phenotype transition also helps brain tissue recovery after stroke[23,62].

GFs play a vital role in the development and survival of endothelial cells, glial cells and neurons[148]. Ischemia would downregulate the level of GFs during the acute phase of stroke[149], which leads to neuron death, and treatment with GFs could protect brain against ischemic injury[150–152]. Moreover, GFs also promoted the compensatory activation and differentiation of NSCs into specific brain cells, and the migration of NSCs to infarcted area, which is in favor of accelerating the recovery of cerebral function at the chronic phase of stroke[153,154]. For instance, administration of nerve growth factor (NGF) promoted the proliferation of NSCs[155], and consecutive treatment enhanced striatal neurogenesis after stroke[156,157]. APOE-modified human serum albumin NPs carrying NGF could assist the peptide penetrate into the damaged brain *via* the interaction between APOE and LRP1, and significantly promoted the brain recovery on two week post-stroke[87]. Superparamagnetic iron oxide encapsulated in APOE-modified albumin NPs could enhance the outcome of magnetic resonance imaging, which could be applied to monitor the structural recovery of brain in real-time.

Recent studies have also shown that multiple microRNAs (miRNAs) are involved in the process of neuronal remodeling, and targeting delivery of miRNAs to mediate neurogenesis is expected to promote the prognosis after stroke[158]. A study has indicated that viral vectors carrying miR-124 could promote angioneurogenesis in mice post-ischemia[159]. However, viral vectors might induce the immune response *in vivo*. Exosomes hold great promise as a gene delivery vector given their unique properties, including low immunogenicity, high delivery efficiency, and inherent ability to cross the BBB[81]. Rabies virus glycoprotein-modified exosomes containing miR-124 could effectively transported miRNA into the ischemic area, and reduced the brain injury by promoting neural precursor cells in the infarcted area to differentiate into neurons[88]. In another study, exosomes containing miR-17-92, obtained from the mesenchymal stromal cells transfected with miR-17-92-contained plasmids could cross the BBB, promote neurogenesis and axon growth, as well as functional recovery after stroke[92].

Table 7 The strategies, including achievements and limitations in targeted delivery of nanomedicines which could regulate inflammation response and promote tissue repair.

Phase of cascade	Target	Nanomedicine and intervention strategy	Achievement	Limitation	Ref.
Inflammation					
Inhibiting pro-inflammatory mediators	Bypass the BBB	Intranasal delivery of e-PAM-R/anti-HMGB1 siRNA complex	Reduced the level of HMGB1 and suppressed the infarct volume	The safety of nasal administration needs further investigation	97
	Passively	CsA-loaded liposomes	Suppressed inflammation and infarct volume	The targeting efficiency need further improvement	53
	Passively	Anti-complement C3 siRNA-loaded PEG-PLA NPs	Decreased the infiltration of inflammatory cells and the level of pro-inflammatory mediators	Conjugating with targeting ligands might improve the treatment effect	55
Regulating inflammatory cells		PEGylated Ce-NPs	Drove microglial polarization from M1 to M2	No experimental data *in vivo*	139
	Fibrin	Rapamycin-loaded micelles modified with fibrin-binding peptide	Promoted the phenotypic transition of microglia from M1 to M2		23
Pretreatment	Passively	TNF-α-loaded PEG-*b*-(PELG-*g*-PLL) NPs	Attenuated the inflammatory activity	The side effects need further study	70
Tissue repair	LRP1	NGF-loaded albumin NPs modified with APOE	Promoted the brain recovery on two weeks post-stroke	The effect of NPs in improving neurological deficits need further evaluation	87
	Biomimetic targeting	miR-124-loaded exosomes modified with rabies virus glycoprotein which could bind the nAChR on BBB	Promoted neural precursor cells in the infarcted area to differentiate into neurons	Neuron-specific targeting ability needs further improvement	88
	Biomimetic targeting	miR-17-92 contained exosomes derived from MSCs	Promoted neurogenesis, axon growth and functional recovery		92
	Biomimetic targeting	Exosomes derived from MSCs	Enhanced neuroprotection and nerve regeneration, improved neurovascular plasticity		162

MSCs, mesenchymal stromal cells; nAChR, nicotinic acetylcholine receptor.

Table 8 The strategies, including achievements and limitations in targeted delivery of nanomedicines which could regulate multiple abnormalities.

Phase of cascade	Target	Nanomedicine and intervention strategy	Achievement	Limitation	Ref.
Combined therapy	Biomimetic targeting and AMT	Nanoplatelets delivering alteplase and neuroprotectant ZL006, TAT-peptide-linked alteplase was conjugated with platelets membrane	Reduced ischemia-related damage in MCAO rats		94
	TfR	Hb and manganous tetroxide NPs-loaded erythrocyte vesicles modified with T7-peptide	Rescued ischemic brain before thrombolysis and after reperfusion	The safety of inorganic NPs needs long-term investigation	161
	CA 1	Glyburide-loaded betulinic acid NPs	Improved the delivery of glyburide to the brain and reduced the oxidative stress	Long term administration might increase the risk of hypoglycemia	77
	NMDAR	NR2B9c-loaded boronic ester dextran NPs coated with RBCs membrane and modified with SHp	Ameliorated oxidative stress and neurological deficit		80
	Anti-firbin antibody	Rapamycin-loaded polymer micelles contained boronic ester group	Ameliorated oxidative stress and promoted the phenotypic transition of microglia from M1 to M2		23

AMT, adsorptive-mediated transport.

Furthermore, exosomes separated from stem cell have been studied for their inherent powerful regenerative ability, as exosomes carry multiple proteins and genes that are related with tissue repair[160]. A large number of evidences have shown that the MSC-derived exosomes possess numerous advantages in the treatment of stroke by enhancing neuroprotection and nerve regeneration, improving neurovascular plasticity, and promoting functional recovery[93]. Therefore, exosomes originating from stem cells could not only deliver drugs to treat stroke, but also enhance the natural process of cerebral repair after injury induced by ischemia. Table 7 summarizes the strategies in targeted delivery of nanomedicines which could regulate inflammation response and promote tissue repair.

5.8. *Nanomedicine regulating multiple abnormalities*

It is worth noting that various events of the ischemic cascade interact with each other. Upstream events will trigger downstream events, and downstream events will in turn amplify upstream events. Therefore, synergistic suppression of multiple abnormalities is expected to achieve better outcomes. Some drugs, such as curcumin, possess anti-oxidation, anti-apoptosis, and anti-inflammatory effects, and thus could simultaneously inhibit multiple abnormalities. In addition, there are many studies that integrated multiple agents with different functions into nanomedicine to realize combined therapy[23,77,80,94,161].

For instance, nanoplatelets were developed to deliver alteplase and neuroprotectant ZL006. In detail, ZL006 was loaded in polymeric NP core, TAT-peptide-linked alteplase was conjugated on the surface of platelets membrane, and the linker could be cleaved by thrombin. NPs coated with platelets membrane retained the thrombus targeting ability of platelets, and released alteplase response to the highly expressed thrombin in clots. Then, the broken of the linker exposed the blocked TAT peptide, which promoted the penetration of NPs across the BBB to deliver ZL006. The combination of thrombolytics and neuroprotectant significantly reduced ischemia-related damage in MCAO rats[94].

In addition to combined treatment with thrombolytics and neuroprotective agents, inhibiting multiple stages of the ischemic

Table 9 Nanomedicines possessing response release ability and the corresponding stimulator.

Stimulator	Nanomedicine	Ref.
Ultrasound	Urokinase-loaded PEG crossed glycol chitosan soft NPs	99,100
Target sensitive	Target sensitive SK-loaded liposomes modified with cRGD	102−104
Phospholipase A2	SK-loaded nanovesicles composed of glycerophospholipids	105
Thrombin	Nanoplatelets conjugated with TAT-peptide-coupled alteplase	94
Thrombin	Thrombin responsive size-shrinkable NPs	76
ROS	Glyburide-loaded betulinic acid NPs	77
ROS	Rapamycin-loaded polymer micelles contained boronic ester group	23
ROS	NR2B9c peptide-loaded NPs contained boronic ester group	80

cascade is also a feasible method. Before reperfusion, hypoxia not only causes neuronal energy depletion, but also leads to the production of ROS; then reperfusion brings a boost of oxygen and leads to a large amount of ROS. T7-peptide-modified erythrocyte vesicles which contain Hb and manganous tetroxide NPs were developed to resolve this problem. The multifunctional vesicles showed superior O_2 release capacity in the damaged brain before thrombolysis, and efficiently inhibited the boost of O_2 after reperfusion, as Hb releases O_2 under hypoxia and stores O_2 in an oxygen-rich environment. In addition, manganous tetroxide NPs exhibited long-lasting scavenging ability against ROS. Regulating the level of O_2 and ROS rescued ischemic brain before thrombolysis and after reperfusion[161].

Moreover, polymer NPs with ROS-sensitive property are used to deliver drugs, which achieved antioxidant therapy through eliminating ROS, and realized combined therapy *via* carrying different drugs. For instance, glyburide-encapsulated betulinic acid NPs[77], NR2B9c peptide-loaded boronic ester-modified dextran NPs[80], and rapamycin-loaded boronic ester-modified polymer micelles[23]. The strategies, including achievement and limitation in targeted delivery of nanomedicine which could regulate multiple abnormalities are summarized in Table 8.

5.9. *The response release of drug in the pathological site*

For targeted delivery of nanomedicines, the release of the drug in response to the microenvironment of lesion could reduce the leakage of the drug in blood, increase the concentration of the drug at the targeting site, and reduce the administrated dosage and side effects. In the treatment of stroke, enzymes that are highly expressed in thrombus, such as thrombin, phospholipase A2, and ROS in ischemic brain tissue are often employed as stimulators of response release. Table 9 summarizes the nanomedicines which possess response release ability and the corresponding stimulators reviewed in this article.

6. Summary and prospect

Due to the lack of effective treatment method, the therapy and long-term recovery of ischemic stroke remains a main challenge for doctors and patients. Recently, nanomedicines have been widely used in the treatment of stroke, as it improved the therapeutic effect of traditional drugs and provided possibilities for the realization of emerging therapies, such as gene therapy[52]. Nanomedicines for targeted delivery of thrombolytics and neuroprotectant have also exhibited good results in preclinical research.

It is worth mentioning that the ischemic cascade events occur at different intervals and interacts with each other[163]. Therefore, a great number of nanomedicines have shown functions against multiple pathological events at different stages of the cascade reaction[62,82]. In theory, intervening the upstream events of ischemic cascade should be suitable for the treatment of stroke, yet some upstream events occur very quickly, even if the interventions were carried out within one or 2 h after ischemic attack, the damage caused by aforementioned events is still difficult to reverse completely[46]. Thus, nanomedicines targeting downstream events of ischemic cascade such as oxidative stress, inflammation response, and tissue repair might be more promising in the treatment of stroke[23,88].

The properties of nanocarrier, including particle size, shape, hydrophilic characteristics and surface charge would affect the fate of nanomedicines *in vivo*. For instance, normally, the half-life of negatively charged NPs *in vivo* is longer, since positively charged NPs tend to absorb proteins such as opsonin in blood. PEGylation would enhance the hydrophilicity of NPs and reduce the opsonizing effect of opsonin, which further reduces the phagocytosis of RES and extends the circulation time of nanomedicine.

In addition, the unique pathophysiological environment of the body will also affect the fate of particles. After ischemia and reperfusion, the integrity of the BBB is destroyed and the permeability increases, nanomedicines could accumulate in the brain through the damaged BBB. Non-targeted nanomedicine might mainly accumulate in the ischemic core where the BBB is disturbed more seriously[60,118], which is not conducive to the rescue of ischemic penumbra. In order to improve the permeability of the BBB, BBB modulators were adopted to temporarily open the tight junction to allow more NPs to penetrate into the brain. The long-term safety of this method still needs further investigation, as excessive opening of the BBB will increase the risk of hemorrhagic transformation.

Modifying nanomedicines with specific ligands through covalent conjugation or electrostatic interaction would further increase the accumulation of NPs in the brain through receptor-mediated transcytosis. After stroke, multiple receptors are highly expressed on BBB, which provides new opportunities for the targeted delivery of nanomedicine[78,80,84]. It is worth noting that targeted nanomedicine modified with endogenous ligand may encounter competition with endogenous ligand in the blood when passing through the BBB, which reduces the targeting efficiency. Fortunately, antibodies with stronger specificity to receptors have been developed, and the interactions are not affected by endogenous ligands.

Among numerous nanomedicines, liposomes, micelles, and polymeric NPs are mainly investigated due to their mature preparation technology, with the hope of translation into clinical application. Although inorganic NPs have favorable uniformity and are easy to be industrialized, the safety needs further serious investigation if used in human. In recent years, biomimetic nanomedicines, based on living cells or cell membrane vesicles/exosomes, have provided new opportunities for drugtargeting delivery. The unique biocompatibility and safety, and inherent targeting properties promote them as a research hotspot of nanomedicines for targeted treatment of stroke, with the expectation of bringing new breakthroughs in stroke treatment[81]. As the development of new carrier and deeper understanding of pathological mechanisms, nanomedicines are casting new lights in stroke treatment.

Acknowledgments

We acknowledge the support from the International Cooperative project of the National Key R&D Program of China (Grant No. 2017YFE0126900), National Natural Science Foundation of China (Grant No. 81872808), Program of Shanghai Academic Research Leader (18XD1400500,China) and Shanghai Municipal Science and Technology Major Project (No.2018SHZDZX01, China) and ZJ Lab.

Author contributions

Chao Li composed the article under the guidance of Tao Sun and Chen Jiang.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics–2020 update: a report from the American heart association. *Circulation* 2020;**141**: e139–96.
2. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2013;**44**: 2064–89.
3. Karaszewski B, Wardlaw JM, Marshall I, Cvoro V, Wartolowska K, Haga K, et al. Early brain temperature elevation and anaerobic metabolism in human acute ischaemic stroke. *Brain* 2009;**132**: 955–64.
4. Castillo J, Loza MI, Mirelman D, Brea J, Blanco M, Sobrino T, et al. A novel mechanism of neuroprotection: blood glutamate grabber. *J Cerebr Blood Flow Metabol* 2016;**36**:292–301.
5. Lewén A, Matz P, Chan PH. Free radical pathways in CNS injury. *J Neurotrauma* 2000;**17**:871–90.
6. Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. *J Cerebr Blood Flow Metabol* 2001;**21**:2–14.
7. Gürsoy-Ozdemir Y, Bolay H, Saribaş O, Dalkara T. Role of endothelial nitric oxide generation and peroxynitrite formation in reperfusion injury after focal cerebral ischemia. *Stroke* 2000;**31**: 1974–80.
8. Peerschke EI, Yin W, Ghebrehiwet B. Complement activation on platelets: implications for vascular inflammation and thrombosis. *Mol Immunol* 2010;**47**:2170–5.
9. Peerschke EI, Reid KB, Ghebrehiwet B. Platelet activation by C1q results in the induction of alpha IIb/beta 3 integrins (GPIIb-IIIa) and the expression of P-selectin and procoagulant activity. *J Exp Med* 1993;**178**:579–87.
10. Huang J, Kim LJ, Mealey R, Marsh Jr HC, Zhang Y, Tenner AJ, et al. Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. *Science* 1999;**285**:595–9.
11. Mocco J, Mack WJ, Ducruet AF, Sosunov SA, Sughrue ME, Hassid BG, et al. Complement component C3 mediates inflammatory injury following focal cerebral ischemia. *Circ Res* 2006;**99**:209–17.
12. Brown GC, Neher JJ. Microglial phagocytosis of live neurons. *Nat Rev Neurosci* 2014;**15**:209–16.
13. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med* 2011;**17**:796–808.
14. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2019;**50**: e344–418.
15. Hacke W, Donnan G, Fieschi C, Kaste M, Kummer R, Broderick JP, et al. Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *Lancet* 2004;**363**:768–74.
16. Frank B, Grotta JC, Alexandrov AV, Bluhmki E, Lyden P, Meretoja A, et al. Thrombolysis in stroke despite contraindications or warnings?. *Stroke* 2013;**44**:727–33.
17. Peters O, Back T, Lindauer U, Busch C, Megow D, Dreier J, et al. Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat. *J Cerebr Blood Flow Metabol* 1998;**18**:196–205.
18. Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. *Int J Stroke* 2009;**4**:461–70.
19. Kevil CG, Oshima T, Alexander B, Coe LL, Alexander JS. H_2O_2-mediated permeability: role of MAPK and occludin. *Am J Physiol Cell Physiol* 2000;**279**:C21–30.
20. Kelly PJ, Morrow JD, Ning MM, Koroshetz W, Lo EH, Terry E, et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the biomarker evaluation for antioxidant therapies in stroke (BEAT-Stroke) study. *Stroke* 2008;**39**:100–4.
21. Chamorro Á, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol* 2016;**15**:869–81.
22. O'Collins VE, Macleod MR, Donnan GA, Horky LL, Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol* 2006;**59**:467–77.
23. Lu YF, Li C, Chen QJ, Liu PX, Guo Q, Zhang Y, et al. Microthrombus-targeting micelles for neurovascular remodeling and enhanced microcirculatory perfusion in acute ischemic stroke. *Adv Mater* 2019;**31**:e1808361.
24. Han L, Li JF, Huang SX, Huang RQ, Liu SH, Hu X, et al. Peptide-conjugated polyamidoamine dendrimer as a nanoscale tumor-targeted T1 magnetic resonance imaging contrast agent. *Biomaterials* 2011;**32**:2989–98.
25. Fabian RH, DeWitt DS, Kent TA. *In vivo* detection of superoxide anion production by the brain using a cytochrome *c* electrode. *J Cerebr Blood Flow Metabol* 1995;**15**:242–7.
26. Yamato M, Egashira T, Utsumi H. Application of *in vivo* ESR spectroscopy to measurement of cerebrovascular ROS generation in stroke. *Free Radic Biol Med* 2003;**35**:1619–31.
27. Melani A, Turchi D, Vannucchi MG, Cipriani S, Gianfriddo M, Pedata F. ATP extracellular concentrations are increased in the rat striatum during *in vivo* ischemia. *Neurochem Int* 2005;**47**: 442–8.
28. Bours MJ, Swennen EL, Virgilio FD, Cronstein BN, Dagnelie PC. Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther* 2006; **112**:358–404.
29. Marsh BJ, Williams-Karnesky RL, Stenzel-Poore MP. Toll-like receptor signaling in endogenous neuroprotection and stroke. *Neuroscience* 2009;**158**:1007–20.
30. Harari OA, Liao JK. NF-κB and innate immunity in ischemic stroke. *Ann N Y Acad Sci* 2010;**1207**:32–40.
31. Sandoval KE, Witt KA. Blood–brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis* 2008;**32**:200–19.
32. Huang J, Upadhyay UM, Tamargo RJ. Inflammation in stroke and focal cerebral ischemia. *Surg Neurol* 2006;**66**:232–45.
33. Yamasaki Y, Matsuo Y, Matsuura N, Onodera H, Itoyama Y, Kogure K. Transient increase of cytokine-induced neutrophil chemoattractant, a member of the interleukin-8 family, in ischemic brain areas after focal ischemia in rats. *Stroke* 1995;**26**: 318–22.
34. Minami M, Satoh M. Chemokines and their receptors in the brain: pathophysiological roles in ischemic brain injury. *Life Sci* 2003;**74**: 321–7.
35. Wang X, Yue TL, Barone FC, Feuerstein GZ. Monocyte chemoattractant protein-1 messenger RNA expression in rat ischemic cortex. *Stroke* 1995;**26**:661–5.
36. Heo JH, Han SW, Lee SK. Free radicals as triggers of brain edema formation after stroke. *Free Radic Biol Med* 2005;**39**:51–70.
37. Rosell A, Cuadrado E, Ortega-Aznar A, Hernández-Guillamon M, Lo EH, Montaner J. MMP-9-positive neutrophil infiltration is associated to blood–brain barrier breakdown and basal lamina type IV collagen degradation during hemorrhagic transformation after human ischemic stroke. *Stroke* 2008;**39**:1121–6.
38. Huang T, Li N, Gao JQ. Recent strategies on targeted delivery of thrombolytics. *Asian J Pharm Sci* 2019;**14**:233–47.

39. Mackman N. Triggers, targets and treatments for thrombosis. *Nature* 2008;**451**:914−8.
40. Mega JL, Simon T. Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments. *Lancet* 2015;**386**:281−91.
41. Sandercock PA, Counsell C, Kane EJ. Anticoagulants for acute ischaemic stroke. *Cochrane Database Syst Rev* 2015;**2015**. Cd000024.
42. Tanswell P, Modi N, Combs D, Danays T. Pharmacokinetics and pharmacodynamics of tenecteplase in fibrinolytic therapy of acute myocardial infarction. *Clin Pharmacokinet* 2002;**41**:1229−45.
43. Campbell BCV, Mitchell PJ, Churilov L, Yassi N, Kleinig TJ, Dowling RJ, et al. Tenecteplase *versus* alteplase before thrombectomy forischemic stroke. *N Engl J Med* 2018;**378**:1573−82.
44. Medcalf RL. Desmoteplase: discovery, insights and opportunities for ischaemic stroke. *Br J Pharmacol* 2012;**165**:75−89.
45. Trotman M, Vermehren P, Gibson CL, Fern R. The dichotomy of memantine treatment for ischemic stroke: dose-dependent protective and detrimental effects. *J Cerebr Blood Flow Metabol* 2015;**35**: 230−9.
46. Horn J, Haan RJ, Vermeulen M, Luiten PG, Limburg M. Nimodipine in animal model experiments of focal cerebral ischemia: a systematic review. *Stroke* 2001;**32**:2433−8.
47. Amemiya S, Kamiya T, Nito C, Inaba T, Kato K, Ueda M, et al. Anti-apoptotic and neuroprotective effects of edaravone following transient focal ischemia in rats. *Eur J Pharmacol* 2005;**516**:125−30.
48. Edaravone Acute Infarction Study Group. Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. *Cerebrovasc Dis* 2003;**15**:222−9.
49. Huang J, Choudhri TF, Winfree CJ, McTaggart RA, Kiss S, Mocco J, et al. Postischemic cerebrovascular E-selectin expression mediates tissue injury in murine stroke. *Stroke* 2000;**31**:3047−53.
50. Fu Y, Zhang NN, Ren L, Yan YP, Sun N, Li YJ, et al. Impact of an immune modulator fingolimod on acute ischemic stroke. *Proc Natl Acad Sci U S A* 2014;**111**:18315−20.
51. Wang XY, Tsuji K, Lee SR, Ning MM, Furie KL, Buchan AM, et al. Mechanisms of hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke. *Stroke* 2004;**35**: 2726−30.
52. Kaviarasi S, Yuba E, Harada A, Krishnan UM. Emerging paradigms in nanotechnology for imaging and treatment of cerebral ischemia. *J Control Release* 2019;**300**:22−45.
53. Partoazar A, Nasoohi S, Rezayat SM, Gilani K, Mehr SE, Amani A, et al. Nanoliposome containing cyclosporine A reduced neuroinflammation responses and improved neurological activities in cerebral ischemia/reperfusion in rat. *Fundam Clin Pharmacol* 2017;**31**:185−93.
54. Warner DS, Sheng HX, Batinić-Haberle I. Oxidants, antioxidants and the ischemic brain. *J Exp Biol* 2004;**207**:3221−31.
55. Wang Y, Li SY, Shen S, Wang J. Protecting neurons from cerebral ischemia/reperfusion injury *via* nanoparticle-mediated delivery of an siRNA to inhibit microglial neurotoxicity. *Biomaterials* 2018;**161**: 95−105.
56. Kim ID, Lim CM, Kim JB, Nam HY, Nam K, Kim SW, et al. Neuroprotection by biodegradable PAMAM ester (e-PAM-R)-mediated HMGB1 siRNA delivery in primary cortical cultures and in the postischemic brain. *J Control Release* 2010;**142**:422−30.
57. Zahednezhad F, Saadat M, Valizadeh H, Zakeri-Milani P, Baradaran B. Liposome and immune system interplay: challenges and potentials. *J Control Release* 2019;**305**:194−209.
58. Han L, Cai Q, Tian DF, Kong DK, Gou XC, Chen ZM, et al. Targeted drug delivery to ischemic stroke *via* chlorotoxin-anchored, lexiscan-loaded nanoparticles. *Nanomedicine* 2016;**12**:1833−42.
59. Al-Ahmady ZS, Jasim D, Ahmad SS, Wong R, Haley M, Coutts G, et al. Selective liposomal transport through blood–brain barrier disruption in ischemic stroke reveals two distinct therapeutic opportunities. *ACS Nano* 2019;**13**:12470−86.
60. Fukuta T, Ishii T, Asai T, Sato A, Kikuchi T, Shimizu K, et al. Treatment of stroke with liposomal neuroprotective agents under cerebral ischemia conditions. *Eur J Pharm Biopharm* 2015;**97**:1−7.
61. Xu HE, Hua Y, Zhong J, Li XL, Xu W, Cai YY, et al. Resveratrol delivery by albumin nanoparticles improved neurological function and neuronal damage in transient middle cerebral artery occlusion rats. *Front Pharmacol* 2018;**9**:1403.
62. Wang Y, Luo J, Li SY. Nano-curcumin simultaneously protects the blood–brain barrier and reduces M1 microglial activation during cerebral ischemia–reperfusion injury. *ACS Appl Mater Interfaces* 2019;**11**:3763−70.
63. Li N, Feng LL, Tan YJ, Xiang Y, Zhang RQ, Yang M. Preparation, characterization, pharmacokinetics and biodistribution of baicalin-loaded liposome on cerebral ischemia–reperfusion after i.v. administration in rats. *Molecules* 2018;**23**:1747−51.
64. Tan LW, Liang C, Wang YY, Jiang Y, Zeng SQ, Tan R. Pharmacodynamic effect of luteolin micelles on alleviating cerebral ischemia reperfusion injury. *Pharmaceutics* 2018;**10**:248.
65. Marushima A, Suzuki K, Nagasaki Y, Yoshitomi T, Toh K, Tsurushima H, et al. Newly synthesized radical-containing nanoparticles enhance neuroprotection after cerebral ischemia–reperfusion injury. *Neurosurgery* 2011;**68**:1418−25.
66. Hosoo H, Marushima A, Nagasaki Y, Hirayama A, Ito H, Puentes S, et al. Neurovascular unit protection from cerebral ischemia–reperfusion injury by radical-containing nanoparticles in mice. *Stroke* 2017;**48**:2238−47.
67. Liu YL, Ai KL, Ji XY, Askhatova D, Du R, Lu LH, et al. Comprehensive insights into the multi-antioxidative mechanisms of melanin nanoparticles and their application to protect brain from injury in ischemicstroke. *J Am Chem Soc* 2017;**139**:856−62.
68. Petro M, Jaffer H, Yang J, Kabu S, Morris VB, Labhasetwar V. Tissue plasminogen activator followed by antioxidant-loaded nanoparticle delivery promotes activation/mobilization of progenitor cells in infarcted rat brain. *Biomaterials* 2016;**81**: 169−80.
69. Kim CK, Kim T, Choi IY, Soh M, Kim D, Kim YJ, et al. Ceria nanoparticles that can protect against ischemic stroke. *Angew Chem Int Ed Engl* 2012;**51**:11039−43.
70. Xu GT, Gu H, Hu B, Tong F, Liu DJ, Yu XJ, et al. PEG-*b*-(PELG-*g*-PLL) nanoparticles as TNF-α nanocarriers: potential cerebral ischemia/reperfusion injury therapeutic applications. *Int J Nanomed* 2017;**12**:2243−54.
71. Jin Q, Cai Y, Li SH, Liu HR, Zhou XY, Lu CQ, et al. Edaravone-encapsulated agonistic micelles rescue ischemic brain tissue by tuning blood–brain barrier permeability. *Theranostics* 2017;**7**:884−98.
72. Han L, Kong DK, Zheng MQ, Murikinati S, Ma C, Yuan P, et al. Increased nanoparticle delivery to brain tumors by autocatalytic priming for improved treatment and imaging. *ACS Nano* 2016;**10**: 4209−18.
73. Lu YF, Jiang C. Brain-targeted polymers for gene delivery in the treatment of brain diseases. *Top Curr Chem* 2017;**375**:48 (Cham).
74. Zhao LX, Liu AC, Yu SW, Wang ZX, Lin XQ, Zhai GX, et al. The permeability of puerarin-loaded poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 on the blood–brain barrier and its protective effect against cerebral ischemia/reperfusion injury. *Biol Pharm Bull* 2013;**36**:1263−70.
75. Ma JN, Zhang SQ, Liu J, Liu FY, Du FY, Li M, et al. Targeted drug delivery to stroke *via* chemotactic recruitment of nanoparticles coated with membrane of engineered neural stem cells. *Small* 2019; **15**:e1902011.
76. Guo X, Deng G, Liu J, Zou P, Du FY, Liu FY, et al. Thrombin-responsive, brain-targeting nanoparticles for improved stroke therapy. *ACS Nano* 2018;**12**:8723−32.
77. Deng G, Ma C, Zhao HT, Zhang SQ, Liu J, Liu FY, et al. Anti-edema and antioxidant combination therapy for ischemic stroke *via* glyburide-loaded betulinic acid nanoparticles. *Theranostics* 2019;**9**: 6991−7002.

78. Wang ZY, Zhao Y, Jiang Y, Lv W, Wu L, Wang BY, et al. Enhanced anti-ischemic stroke of ZL006 by T7-conjugated PEGylated liposomes drug delivery system. *Sci Rep* 2015;**5**:12651.
79. Zhao Y, Jiang Y, Lv W, Wang ZY, Lv LY, Wang BY, et al. Dual targeted nanocarrier for brain ischemic stroke treatment. *J Control Release* 2016;**233**:64−71.
80. Lv W, Xu JP, Wang XQ, Li XR, Xu QW, Xin HL. Bioengineered boronic ester modified dextran polymer nanoparticles as reactive oxygen species responsive nanocarrier for ischemic stroke treatment. *ACS Nano* 2018;**12**:5417−26.
81. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* 2018;**150**:137−49.
82. Yun X, Maximov VD, Yu J, Zhu H, Vertegel AA, Kindy MS. Nanoparticles for targeted delivery of antioxidant enzymes to the brain after cerebral ischemia and reperfusion injury. *J Cerebr Blood Flow Metabol* 2013;**33**:583−92.
83. Zhang T, Li CY, Jia JJ, Chi JS, Zhou D, Li JZ, et al. Combination therapy with LXW7 and ceria nanoparticles protects against acute cerebral ischemia/reperfusion injury in rats. *Curr Med Sci* 2018;**38**:144−52.
84. Bao QQ, Hu P, Xu YY, Cheng TS, Wei CY, Pan LM, et al. Simultaneous blood–brain barrier crossing and protection for stroke treatment based on edaravone-loaded ceria nanoparticles. *ACS Nano* 2018;**12**:6794−805.
85. Ishii T, Asai T, Oyama D, Fukuta T, Yasuda N, Shimizu K, et al. Amelioration of cerebral ischemia–reperfusion injury based on liposomal drug delivery system with asialo-erythropoietin. *J Control Release* 2012;**160**:81−7.
86. Ishii T, Asai T, Fukuta T, Oyama D, Yasuda N, Agato Y, et al. A single injection of liposomal asialo-erythropoietin improves motor function deficit caused by cerebral ischemia/reperfusion. *Int J Pharm* 2012;**439**:269−74.
87. Feczkó T, Piiper A, Ansar S, Blixt FW, Ashtikar M, Schiffmann S, et al. Stimulating brain recovery after stroke using theranostic albumin nanocarriers loaded with nerve growth factor in combination therapy. *J Control Release* 2019;**293**:63−72.
88. Yang JL, Zhang XF, Chen XJ, Wang L, Yang GD. Exosome-mediated delivery of miR-124 promotes neurogenesis after ischemia. *Mol Ther Nucleic Acids* 2017;**7**:278−87.
89. Hu CM, Zhang L, Aryal S, Cheung C, Fang RH, Zhang LF. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proc Natl Acad Sci U S A* 2011;**108**:10980−5.
90. Hu CM, Fang RH, Wang KC, Luk BT, Thamphiwatana S, Dehaini D, et al. Nanoparticle biointerfacing by platelet membrane cloaking. *Nature* 2015;**526**:118−21.
91. Zhang C, Ling CL, Pang L, Wang Q, Liu JX, Wang BS, et al. Direct macromolecular drug delivery to cerebral ischemia area using neutrophil-mediated nanoparticles. *Theranostics* 2017;**7**:3260−75.
92. Xin HQ, Katakowski M, Wang FJ, Qian JY, Liu XS, Ali MM, et al. MicroRNA cluster miR-17-92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. *Stroke* 2017;**48**:747−53.
93. Xin HQ, Li Y, Cui YS, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. *J Cerebr Blood Flow Metabol* 2013;**33**:1711−5.
94. Xu JP, Wang XQ, Yin HY, Cao X, Hu QY, Lv W, et al. Sequentially site-specific delivery of thrombolytics and neuroprotectant for enhanced treatment of ischemic stroke. *ACS Nano* 2019;**13**:8577−88.
95. Liu X, An CY, Jin P, Liu XS, Wang LH. Protective effects of cationic bovine serum albumin-conjugated PEGylated tanshinone IIA nanoparticles on cerebral ischemia. *Biomaterials* 2013;**34**:817−30.
96. Kalani A, Chaturvedi P, Kamat PK, Maldonado C, Bauer P, Joshua IG, et al. Curcumin-loaded embryonic stem cell exosomes restored neurovascular unit following ischemia–reperfusion injury. *Int J Biochem Cell Biol* 2016;**79**:360−9.
97. Kim ID, Shin JH, Kim SW, Choi S, Ahn J, Han PL, et al. Intranasal delivery of HMGB1 siRNA confers target gene knockdown and robust neuroprotection in the postischemic brain. *Mol Ther* 2012;**20**:829−39.
98. Harston GW, Okell TW, Sheerin F, Schulz U, Mathieson P, Reckless I, et al. Quantification of serial cerebral blood flow in acute stroke using arterial spin labeling. *Stroke* 2017;**48**:123−30.
99. Jin HQ, Tan H, Zhao LL, Sun WP, Zhu LJ, Sun YA, et al. Ultrasound-triggered thrombolysis using urokinase-loaded nanogels. *Int J Pharm* 2012;**434**:384−90.
100. Teng YM, Jin HQ, Nan D, Li MN, Fan CH, Liu YY, et al. *In vivo* evaluation of urokinase-loaded hollow nanogels for sonothrombolysis on suture embolization-induced acute ischemic stroke rat model. *Bioact Mater* 2018;**3**:102−9.
101. Yurko Y, Maximov V, Andreozzi E, Thompson GL, Vertegel AA. Design of biomedical nanodevices for dissolution of blood clots. *Mater Sci Eng C* 2009;**29**:737−41.
102. Vaidya B, Nayak MK, Dash D, Agrawal GP, Vyas SP. Development and characterization of site specific target sensitive liposomes for the delivery of thrombolytic agents. *Int J Pharm* 2011;**403**:254−61.
103. Vaidya B, Agrawal GP, Vyas SP. Platelets directed liposomes for the delivery of streptokinase: development and characterization. *Eur J Pharm Sci* 2011;**44**:589−94.
104. Vaidya B, Nayak MK, Dash D, Agrawal GP, Vyas SP. Development and characterization of highly selective target-sensitive liposomes for the delivery of streptokinase: in vitro/*in vivo* studies. *Drug Deliv* 2016;**23**:801−7.
105. Pawlowski CL, Li W, Sun M, Ravichandran K, Hickman D, Kos C, et al. Platelet microparticle-inspired clot-responsive nanomedicine for targeted fibrinolysis. *Biomaterials* 2017;**128**:94−108.
106. Saxena R, Wijnhoud AD, Carton H, Hacke W, Kaste M, Przybelski RJ, et al. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. *Stroke* 1999;**30**:993−6.
107. Bobofchak KM, Mito T, Texel SJ, Bellelli A, Nemoto M, Traystman RJ, et al. A recombinant polymeric hemoglobin with conformational, functional, and physiological characteristics of an *in vivo* O_2 transporter. *Am J Physiol Heart Circ Physiol* 2003;**285**:H549−61.
108. Kawaguchi AT, Fukumoto D, Haida M, Ogata Y, Yamano M, Tsukada H. Liposome-encapsulated hemoglobin reduces the size of cerebral infarction in the rat: evaluation with photochemically induced thrombosis of the middle cerebral artery. *Stroke* 2007;**38**:1626−32.
109. Fukumoto D, Kawaguchi AT, Haida M, Yamano M, Ogata Y, Tsukada H. Liposome-encapsulated hemoglobin reduces the size of cerebral infarction in rats: effect of oxygen affinity. *Artif Organs* 2009;**33**:159−63.
110. Hamadate N, Yamaguchi T, Sugawara A, Togashi H, Izumi T, Yoshida T, et al. Liposome-encapsulated hemoglobin ameliorates impairment of fear memory and hippocampal dysfunction after cerebral ischemia in rats. *J Pharmacol Sci* 2010;**114**:409−19.
111. Komatsu H, Furuya T, Sato N, Ohta K, Matsuura A, Ohmura T, et al. Effect of hemoglobin vesicle, a cellular-type artificial oxygen carrier, on middle cerebral artery occlusion- and arachidonic acid-induced stroke models in rats. *Neurosci Lett* 2007;**421**:121−5.
112. Simard JM, Chen MK, Tarasov KV, Bhatta S, Ivanova S, Melnitchenko L, et al. Newly expressed SUR1-regulated NC(Ca-ATP) channel mediates cerebral edema after ischemic stroke. *Nat Med* 2006;**12**:433−40.
113. Simard JM, Sheth KN, Kimberly WT, Stern BJ, Zoppo GJ, Jacobson S, et al. Glibenclamide in cerebral ischemia and stroke. *Neurocrit Care* 2014;**20**:319−33.
114. Pergakis M, Badjatia N, Chaturvedi S, Cronin CA, Kimberly WT, Sheth KN, et al. BIIB093 (IV glibenclamide): an investigational compound for the prevention and treatment of severe cerebral edema. *Expet Opin Invest Drugs* 2019;**28**:1031−40.

115. Tournier N, Saba W, Cisternino S, Peyronneau MA, Damont A, Goutal S, et al. Effects of selected OATP and/or ABC transporter inhibitors on the brain and whole-body distribution of glyburide. *AAPS J* 2013;**15**:1082−90.
116. Zhou L, Li F, Xu HB, Luo CX, Wu HY, Zhu MM, et al. Treatment of cerebral ischemia by disrupting ischemia-induced interaction of nNOS with PSD-95. *Nat Med* 2010;**16**:1439−43.
117. Liu SH, Guo YB, Huang RQ, Li JF, Huang SX, Kuang YY, et al. Gene and doxorubicin co-delivery system for targeting therapy of glioma. *Biomaterials* 2012;**33**:4907−16.
118. Ishii T, Asai T, Oyama D, Agato Y, Yasuda N, Fukuta T, et al. Treatment of cerebral ischemia–reperfusion injury with PEGylated liposomes encapsulating FK506. *FASEB J* 2013;**27**:1362−70.
119. Cao YG, Mao XY, Sun CY, Zheng P, Gao JQ, Wang XR, et al. Baicalin attenuates global cerebral ischemia/reperfusion injury in gerbils *via* anti-oxidative and anti-apoptotic pathways. *Brain Res Bull* 2011;**85**:396−402.
120. Qiao HM, Dong LP, Zhang XJ, Zhu CH, Zhang XL, Wang LN, et al. Protective effect of luteolin in experimental ischemic stroke: upregulated SOD1, CAT, BCL-2 and claudin-5, down-regulated MDA and Bax expression. *Neurochem Res* 2012;**37**:2014−24.
121. Doggui S, Sahni JK, Arseneault M, Dao L, Ramassamy C. Neuronal uptake and neuroprotective effect of curcumin-loaded PLGA nanoparticles on the human SK-N-SH cell line. *J Alzheimers Dis* 2012;**30**:377−92.
122. Djiokeng Paka G, Doggui S, Zaghmi A, Safar R, Dao L, Reisch A, et al. Neuronal uptake and neuroprotective properties of curcumin-loaded nanoparticles on SK-N-SH cell line: role of poly(lactide-*co*-glycolide) polymeric matrix composition. *Mol Pharm* 2016;**13**:391−403.
123. Qiu JF, Gao X, Wang BL, Wei XW, Gou ML, Men K, et al. Preparation and characterization of monomethoxy poly(ethylene glycol)-poly(*ε*-caprolactone) micelles for the solubilization and *in vivo* delivery of luteolin. *Int J Nanomed* 2013;**8**:3061−9.
124. Yoshitomi T, Suzuki R, Mamiya T, Matsui H, Hirayama A, Nagasaki Y. pH-sensitive radical-containing-nanoparticle (RNP) for the L-band-EPR imaging of low pH circumstances. *Bioconjugate Chem* 2009;**20**:1792−8.
125. Yoshitomi T, Miyamoto D, Nagasaki Y. Design of core-shell-type nanoparticles carrying stable radicals in the core. *Biomacromolecules* 2009;**10**:596−601.
126. Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH. Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. *Proc Natl Acad Sci U S A* 1991;**88**:11158−62.
127. Armogida M, Spalloni A, Amantea D, Nutini M, Petrelli F, Longone P, et al. The protective role of catalase against cerebral ischemia *in vitro* and *in vivo*. *Int J Immunopathol Pharmacol* 2011;**24**:735−47.
128. Reddy MK, Labhasetwar V. Nanoparticle-mediated delivery of superoxide dismutase to the brain: an effective strategy to reduce ischemia–reperfusion injury. *FASEB J* 2009;**23**:1384−95.
129. Huang X, Ding J, Li YF, Liu WJ, Ji JL, Wang H, et al. Exosomes derived from PEDF modified adipose-derived mesenchymal stem cells ameliorate cerebral ischemia–reperfusion injury by regulation of autophagy and apoptosis. *Exp Cell Res* 2018;**371**:269−77.
130. Sirén AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, et al. Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci U S A* 2001;**98**:4044−9.
131. Erbayraktar S, Grasso G, Sfacteria A, Xie QW, Coleman T, Kreilgaard M, et al. Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity *in vivo*. *Proc Natl Acad Sci U S A* 2003;**100**:6741−6.
132. Sanchez A, Tripathy D, Yin X, Luo J, Martinez J, Grammas P. Pigment epithelium-derived factor (PEDF) protects cortical neurons in vitro from oxidant injury by activation of extracellular signal-regulated kinase (ERK) 1/2 and induction of BCL-2. *Neurosci Res* 2012;**72**:1−8.
133. Wang Q, Gou XC, Xiong LZ, Jin WL, Chen SY, Hou LC, et al. Trans-activator of transcription-mediated delivery of NEP1-40 protein into brain has a neuroprotective effect against focal cerebral ischemic injury *via* inhibition of neuronal apoptosis. *Anesthesiology* 2008;**108**:1071−80.
134. Chamorro Á, Meisel A, Planas AM, Urra X, Beek DV, Veltkamp R. The immunology of acute stroke. *Nat Rev Neurol* 2012;**8**:401−10.
135. Castillo J, Moro MA, Blanco M, Leira R, Serena J, Lizasoain I, et al. The release of tumor necrosis factor-alpha is associated with ischemic tolerance in human stroke. *Ann Neurol* 2003;**54**:811−9.
136. Zhao SC, Ma LS, Chu ZH, Xu H, Wu WQ, Liu FD. Regulation of microglial activation in stroke. *Acta Pharmacol Sin* 2017;**38**:445−58.
137. Xia CY, Zhang S, Gao Y, Wang ZZ, Chen NH. Selective modulation of microglia polarization to M2 phenotype for stroke treatment. *Int Immunopharm* 2015;**25**:377−82.
138. Hu XM, Li PY, Guo YL, Wang HY, Leak RK, Chen S, et al. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* 2012;**43**:3063−70.
139. Zeng F, Wu YW, Li XW, Ge XJ, Guo QH, Lou XB, et al. Custom-made ceria nanoparticles show a neuroprotective effect by modulating phenotypic polarization of the microglia. *Angew Chem Int Ed Engl* 2018;**57**:5808−12.
140. Wong GH, Goeddel DV. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* 1988;**242**:941−4.
141. Hallenbeck JM. The many faces of tumor necrosis factor in stroke. *Nat Med* 2002;**8**:1363−8.
142. Ginis I, Jaiswal R, Klimanis D, Liu J, Greenspon J, Hallenbeck JM. TNF-alpha-induced tolerance to ischemic injury involves differential control of NF-kappaB transactivation: the role of NF-kappaB association with p300 adaptor. *J Cerebr Blood Flow Metabol* 2002;**22**:142−52.
143. Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM. TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. *J Cerebr Blood Flow Metabol* 1997;**17**:483−90.
144. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. *Circ Res* 2010;**107**:1170−84.
145. Schilling M, Besselmann M, Müller M, Strecker JK, Ringelstein EB, Kiefer R. Predominant phagocytic activity of resident microglia over hematogenous macrophages following transient focal cerebral ischemia: an investigation using green fluorescent protein transgenic bone marrow chimeric mice. *Exp Neurol* 2005;**196**:290−7.
146. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci* 2005;**8**:752−8.
147. Nathan C, Ding AH. Nonresolving inflammation. *Cell* 2010;**140**:871−82.
148. Abe K. Therapeutic potential of neurotrophic factors and neural stem cells against ischemic brain injury. *J Cerebr Blood Flow Metabol* 2000;**20**:1393−408.
149. Lee TH, Kato H, Chen ST, Kogure K, Itoyama Y. Expression of nerve growth factor and trkA after transient focal cerebral ischemia in rats. *Stroke* 1998;**29**:1687−96.
150. Holtzman DM, Sheldon RA, Jaffe W, Cheng Y, Ferriero DM. Nerve growth factor protects the neonatal brain against hypoxic-ischemic injury. *Ann Neurol* 1996;**39**:114−22.
151. Shigeno T, Mima T, Takakura K, Graham DI, Kato G, Hashimoto Y, et al. Amelioration of delayed neuronal death in the hippocampus by nerve growth factor. *J Neurosci* 1991;**11**:2914−9.
152. Kitagawa H, Hayashi T, Mitsumoto Y, Koga N, Itoyama Y, Abe K. Reduction of ischemic brain injury by topical application of glial cell line-derived neurotrophic factor after permanent middle cerebral artery occlusion in rats. *Stroke* 1998;**29**:1417−22.
153. Takagi Y, Nozaki K, Takahashi J, Yodoi J, Ishikawa M, Hashimoto N. Proliferation of neuronal precursor cells in the dentate gyrus is accelerated after transient forebrain ischemia in mice. *Brain Res* 1999;**831**:283−7.

154. Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, Kooy DV. *In vivo* growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J Neurosci* 1996;**16**:2649−58.
155. Zhao Y, Xie P, Zhu XF, Cai ZY. Neural stem cell transplantation and nerve growth factor promote neurological recovery in rats with ischemic stroke. *Nan Fang Yi Ke Da Xue Xue Bao* 2008;**28**:1123−6.
156. Zhu WS, Cheng SM, Xu GL, Ma MM, Zhou ZM, Liu DZ, et al. Intranasal nerve growth factor enhances striatal neurogenesis in adult rats with focal cerebral ischemia. *Drug Deliv* 2011;**18**:338−43.
157. Rhim T, Lee M. Targeted delivery of growth factors in ischemic stroke animal models. *Expet Opin Drug Deliv* 2016;**13**:709−23.
158. Liu XS, Chopp M, Zhang RL, Zhang ZG. MicroRNAs in cerebral ischemia-induced neurogenesis. *J Neuropathol Exp Neurol* 2013;**72**:718−22.
159. Doeppner TR, Doehring M, Bretschneider E, Zechariah A, Kaltwasser B, Müller B, et al. MicroRNA-124 protects against focal cerebral ischemia *via* mechanisms involving Usp14-dependent REST degradation. *Acta Neuropathol* 2013;**126**:251−65.
160. Xin HQ, Li Y, Chopp M. Exosomes/miRNAs as mediating cell-based therapy of stroke. *Front Cell Neurosci* 2014;**8**:377.
161. Shi JJ, Yu WY, Xu LH, Yin N, Liu W, Zhang KX, et al. Bioinspired nanosponge for salvaging ischemic stroke *via* free radical scavenging and self-adapted oxygen regulating. *Nano Lett* 2020;**20**:780−9.
162. Otero-Ortega L, Gómez de Frutos MC, Laso-García F, Rodríguez-Frutos B, Medina-Gutiérrez E, López JA, et al. Exosomes promote restoration after an experimental animal model of intracerebral hemorrhage. *J Cerebr Blood Flow Metabol* 2018;**38**:767−79.
163. Lipton P. Ischemic cell death in brain neurons. *Physiol Rev* 1999;**79**:1431−568.