Nanomedicine for Ischemic Stroke

Subjects: Pathology

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Stroke is a severe brain disease leading to disability and death. Ischemic stroke dominates in stroke cases, and there are no effective therapies in clinic, partly due to the challenges in delivering therapeutics to ischemic sites in the brain. This review is focused on the current knowledge of pathogenesis in ischemic stroke, and its potential therapies and diagnostics. Furthermore, we present recent advances in developments of nanoparticle-based therapeutics for improved treatment of ischemic stroke using polymeric NPs, liposomes and cell-derived nanovesicles. We also address several critical questions in ischemic stroke, such as understanding how nanoparticles cross the blood brain barrier and developing in vivo imaging technologies to address this critical question. Finally, we discuss new opportunities in developing novel therapeutics by targeting activated brain endothelium and inflammatory neutrophils to improve the current therapies for ischemic stroke.

Keywords: ischemic stroke ; blood brain barrier ; nanoparticle-based drug delivery ; brain targeting

1. Introduction

Stroke is an unexpected and acute brain disease. It is reported that one of nineteen deaths is related to stroke in the United States, and the mortality rate of stroke is as high as 30% [1]. Stroke is defined by a condition caused by a hemorrhage or occlusion of cerebral blood vessels. Lacking of blood flow in the brain causes dysfunctions of brain cells, oxidative stress, and neurological damage [2]. The symptoms of stroke include numbness, confusion, and aphasia, and those signs are related to injured areas in the brain [3]. Since 87% of strokes are related to ischemia in the brain and 13% are involved with the hemorrhage, this review will focus on discussing how nanotechnology improves therapies and diagnosis of ischemia stroke.

Cerebral ischemia initiates a cascade of pathological processes, eventually causing neuron death. Reperfusion is a clinical method to restore the blood flow in the brain by administration of tissue plasminogen activator (t-PA) or mechanical thrombectomy (MT) for treatment of ischemic stroke [4]. However, reperfusion often leads to tissue damage because oxygen influx of reperfusion generates reactive oxygen species (ROS), initiating inflammatory responses including cytokine production and leukocyte infiltration [5]. Many neuroprotectants have been developed to alleviate reperfusion-induced injury, but none of them were clinically approved. There are several reasons for this failure: (1) ineffective drug delivery into the brain because of blood brain barrier (BBB), (2) drugs with short circulation times, poor stability, and toxicity, (3) difficulty in choosing right drugs and doses due to the heterogenicity of stroke (e.g., disease locations and severity).

Recently, nanotechnology emerges as innovative tools in drug delivery and diagnosis to treat a wide range of diseases, such as cancer and inflammatory disorders [6][7][8][9][10][11][12][13]. In the case of ischemic stroke, nanoparticles could possibly deliver therapeutics across BBB, prolong the drug circulation, and increase the drug accumulation at diseased sites. In addition, nanoparticles can be utilized as innovative diagnostic systems to detect several biomarkers (such as ROS and neurotransmitters) in the brain for early-stage stroke diagnosis. In this review, we firstly describe the physiopathology of ischemic stroke and current limitations in therapy and diagnosis. Then, we discuss how nanotechnology offers opportunities in treating ischemic stroke, highlighting the recent progress on delivering neuroprotective agents, anti-inflammatory drugs, and small interfering RNA (siRNA) to alleviate ischemic injuries. We also review the applications of nanomaterials in stroke diagnosis. Finally, we describe new opportunities in translating nanoparticle-based therapies to clinic in the prevention and treatment of ischemic stroke.

2. Stroke Treatment and Diagnosis using Nanotechnology

Nanoparticles are smaller than a cell and larger than small molecules; thus, nanoparticles are excellent carriers to deliver therapeutics (drugs) and control their release for improved therapies in a wide range of diseases [42–44]. Nanoparticle-based drug delivery systems may solve the current challenges in ischemic stroke treatment. Ishii and co-workers

developed asialo-erythropoietin (AEPO)-modified PEGylated liposomes (AEPO-liposomes) to treat cerebral I/R injury [48]. Asialo-EPO (AEPO) is a neuroprotective agent that binds to EPO receptor (EPOR) on neuronal cells and activates MAPK and PI3K/Akt pathways for improved outcome of cerebral stroke. The results showed that AEPO-liposomes accumulated in the ischemic lesion for more than 24 h after injection decreased infarct lesions, reduced the neuronal apoptosis, and ameliorated cerebral I/R injury in rats. Nanoparticles were also bioengineered with ROS-responsive features that can control drug release in the ischemic brain. In a study [56], Lv et al. designed nanoparticles to deliver NR2B9C (a neuroprotectant agent) to treat ischemic stroke. Nanoparticles (named SHp-RBC-NPs) composed of a red blood cell (RBC) membrane as a shell and a polymer nanoparticle with ROS-responsive boronic ester as a core. To target the ischemic brain, a peptide, SHp (CLEVSRKNC), was conjugated on the surface of nanoparticles. Triggered by high levels of ROS in ischemic region, the nanoparticles can control the release of NR2B9C in ischemic brain tissues. Ex vivo brain fluorescence imaging showed that SHp-RBC-NP can target the ischemic brain to significantly prevent neurological damage and reduce the brain infarction size. Dong et al. reported neutrophil cell membrane-derived nanovesicles (HVs), which can target inflamed endothelium at I/R injury sites and deliver therapeutics to treat the mouse I/R injury [68]. The nanovesicles were generated from differentiated HL-60 cells (neutrophil-like cells) using nitrogen cavitation. The nitrogen cavitation approach was used to disrupt cell membrane to eliminate nuclei and cytosols of cells. The TEM image showed the liposome-like structure of nanovesicles made from HL-60 cells and the size was 200 nm in diameter. In vivo imaging system (IVIS) and confocal microscopy showed that HVs (nanovesicles made from differentiated HL-60 cells) were specifically accumulated in the injured half of the brain rather than in the normal brain. To visualize how HVs interacted with brain vasculature, a cranial window was established and intravital microscopy was performed in live mice . The real-time visualization images strongly indicated that HVs can specifically target ischemic vasculature. To examine the delivery of therapeutics with nanovesicles, Resolvin D2 (RvD2) was loaded into the membrane of nanovesicles because RvD2 is a new lipid mediator to resolve inflammatory responses [84]. After Resolvin D2-loaded nanovesicles (RvD2-HVs) were intravenously injected into mice, reduced neutrophil infiltration was observed using intravital microcopy in live mice, and brain homogenates also confirmed this observation . Subsequently, the level cytokines, such as TNF-α, were decreased after RvD2-HVs were administered. The data indicated that RvD2-HVs could alleviate inflammation responses in ischemic stroke. The diminished inflammatory responses reduced the infarction sizes and prevented neurological damage from ischemic stroke.

In terms of nanotechnology in stroke diagnosis, Shen's research groups developed a method to track administered stem cells and investigated cell-based therapies in ischemic stroke. Cationic polymersomes formed polymeric vesicles and they were loaded with superparamagnetic iron oxide nanoparticles (SPIONs) and quantum dots (QDs) as imaging agents. Cationic polymersome vesicles were incubated with neural stem cells (NSCs) to label the NSCs, thus NSCs could be tracked after they were injected to the striatum contralateral of the ischemic hemisphere. The migration and location of NSCs were monitored using MRI imaging in six weeks in a rat MCAO model and the optical imaging tracked the cells for four weeks [14]. Another example is that neural stem cells (NSCs) were labeled with an MRI reporter ferritin heavy chain (FTH) and enhanced green fluorescent protein (EGFP) to monitor the stem cells in the long-term for detecting ischemic stroke [15]. Those approaches are promising to understand how stem cells target the ischemic tissues and to develop the cell-based therapies to treat ischemic stroke. Andreas et al. conducted a clinical phase II pilot trial using ultra small superparamagnetic iron oxide (USPIO)-enhanced MRI for macrophage imaging since USPIO particles have been introduced as a cell-specific MRI contrast agents taken up by macrophages. USPIO contrast agent was infused in ten patients 5 to 6 days after stroke onset, and MRI was performed within 24–36 h or 48–72 h after the infusion. Results showed that USPIO was much better than gadolinium (a clinically used imaging agent). The study indicates that USPIO-enhanced MRI may provide an in vivo tool to track cells in stroke and other CNS pathologies [16].

3. New Opportunities and Perspectives

Stroke is an acute disease, therefore treating stroke requires the early diagnosis and immediate therapies. This review has highlighted the recent advances in design and engineering of new nanomaterials to target ischemic stroke tissues and new technologies used to image and monitor the disease progression in vivo.

Specifically delivering therapeutics to the injured brain is essential in treating ischemic stroke. While many nanoparticle-based formulations or cell-based platforms have been developed, the fundamental question of how they target ischemic stroke lesion has not been clearly addressed. For example, BBB is the blood vessel barrier to prevent therapeutics across the blood vessels. Most studies showed the dramatic therapeutic effects of using nanoparticle-based formulations compared to free drugs. The enhanced outcomes claimed that nanoparticles transported therapeutics across BBB, but the direct in vivo experimental data were vague to support this conclusion. Developing advanced in vivo imaging systems [17] [18] is needed to visualize the intact brain [19] and to address whether and how nanoparticles cross the BBB.

Brain vasculature during stroke shows the temporal opening, and this disruption of BBB may be a target to guide small molecules across the BBB. Further investigation is needed on the time course of BBB opening to design ideal drug delivery platforms. Although ischemic stroke increases brain vascular permeability, the endothelial gaps are unlikely to allow the efficient transport of nanoparticles because their size is usually larger than endothelial gaps. Therefore, developing new and novel concepts is needed to solve the drug delivery across BBB. Ischemic stroke and reperfusion cause acute inflammatory responses including neutrophil infiltration across blood vessels. Recent studies have shown that rational design of nanoparticles (gold nanoparticles, polymeric nanoparticles, or protein nanoparticles) enables neutrophils to transport nanoparticles across the blood vessel barrier in infection and cancer mouse models [6][20][21][22][23]. It is expected that this technology of hijacking neutrophils in vivo may transport nanotherapeutics across BBB for therapies of ischemic stroke.

Ischemic stroke is strongly correlated to inflammatory responses. Inflammatory responses include endothelial activation and neutrophil infiltration, which damages the brain tissues. Targeting activated endothelium using cell membrane-derived nanovesicles has demonstrated the value in delivering therapeutics to treat ischemic stroke [19]. To translate cell-derived nanovesicles, developing new technologies is needed to scale up their production. Recent studies show that nitrogen cavitation methods [24] and other approaches [25] could have the potential to scale up cell-derived nanovesicles for clinical applications. In addition, Dong [24] et al. reported an interesting study to deliver lipid mediators [26] (such as Resolvin D2) to treat ischemic stroke. This study is different from current therapies that mainly deliver anti-inflammatory agents. Anti-inflammatory therapies can cause side effects [27], but Resolvin D2 is a new drug to increase the host immune defense via increased neutrophil apoptosis and macrophage phagocytosis [28]. In the future, it is needed to investigate how to efficiently load lipid mediators in nanovesicles [29] for improved treatment of ischemic stroke. Another direction is the design of new nanoparticles in response to inflammatory environments (such as pH or enzymes) [8] to improve the treatment of ischemic stroke.

Targeting inflammatory neutrophils in situ to block brain neutrophil infiltration is a new opportunity to treat ischemic stroke. A recent study [7] shows that albumin protein-formed nanoparticles loaded with doxorubicin could induce neutrophil apoptosis, thus inhibiting neutrophil infiltration to prevent brain damage in a mouse ischemic stroke model. This is an exciting and new research area to develop novel therapies to solve the lacking pharmacological therapies for ischemic stroke in clinic.

In addition, developing new drugs that can target inflammatory pathways for management of the host injury during ischemic stroke is needed. The pathogenesis of ischemic stroke is complicated, and it is involved with multiple signaling pathways. The molecular mechanisms of ischemic stroke-induced brain injury are needed to be further determined. The timing of administering drugs or nanoparticle-based therapeutics is also very critical. Optimizing therapeutic windows in the future is needed.

Theranostics formulations are interesting and promising in treating ischemic stroke since they combine diagnosis and therapies. Nanoparticle-based platforms are novel constructs because they can contain imaging agents and drugs in single nanoparticle platforms. For instance, formulations with both neuroprotectants and Fe_2O_3 magnetic nanoparticles can achieve the therapy and imaging. In the future, developing more similar drug delivery systems is needed to treat ischemic stroke. However, considering that many inorganic materials do not naturally exist in the body (although iron oxide nanoparticles (IONPs) have been approved by the US food and drug administration (FDA) to treat anemia), fully evaluating the biodistribution and toxicity after systemic administration is required.

4. Conclusions

It is essential to understand the pathogenesis of ischemic stroke and how nanoparticles interact with ischemic tissues. The fundamental question on how nanoparticles transport therapeutics across BBB is yet to be addressed, and advances in design and synthesis of nanoparticles and novel in vivo imaging systems (such as intravital microscopy) may address this question. The development of new drugs and novel nanoparticle-based therapeutics will improve the outcomes in treating ischemic stroke patients.

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