Nanomaterials in Stroke Treatment
Perspectives

Natallia Shcharbina, PhD; Dzmitry Shcharbin, PhD; Maria Bryszewska, PhD, DSci, Prof

Strok"es the world’s second leading cause of mortality, with a high incidence of severe morbidity in surviving victims. About 87% of strokes are caused by ischemia, and the remainder is caused by hemorrhage. Ischemic stroke causes most deaths.1 Acute ischemic stroke results from a sudden decrease or loss of blood flow in cerebral arteries. Brain tissue has a relatively high consumption of oxygen and glucose, and depends almost exclusively on oxidative phosphorylation for energy production. Cerebral ischemia leads to the development of a cascade of pathological biochemical reactions, including the rapid depletion of the intracellular ATP pool, anaerobic glycolysis, lactate acidosis and membrane depolarization, glutamate excitotoxicity, the entry of Ca2+, Na+, and H2O into cells, activation of Ca-dependent proteins, mitochondrial dysfunction, overproduction of free radicals, activation of the immune system, gene overexpression, and, finally, cell death.1–3 Oxidative stress is one of the main mechanisms of stroke development. Free radical production and the activation of degradative enzymes lead to acute cell death through necrosis, but excitotoxic mechanisms can also initiate apoptosis.4 Besides causing cerebral cellular injury, oxidative stress also increases blood–brain barrier dysfunction.4–5

Stroke Treatment

Major approaches developed to treat acute ischemic stroke fall into 2 categories, early recanalization (thrombolysis) and neuroprotection. There are ongoing trials aimed at evaluating the effectiveness of recombinant tissue–type plasminogen activator and acetylsalicylic acid in longer time windows with a finer selection of patients based on MRI and trials of novel recanalization methods.6 Neuroprotection is achieved by blocking of proinflammatory cytokines and cell adhesion agents, decrease of lipid peroxidation processes, and blocking of apoptosis.7 At least 25 clinical phase II and III trials are currently recruiting patients for the evaluation of new therapeutics for acute ischemic stroke.4 Despite these developments, the actual therapeutic arsenal for acute ischemic stroke is highly limited, and no new treatment has proven efficacious and safe in randomized clinical trials to date.8 The clinical failure of most neuroprotectors studied can be explained as follows. First, in clinical units, stroke treatment is often initiated outside the therapeutic window of the drug proposed. Second, many effective neuroprotectors cannot penetrate through the blood–brain barrier into the cerebral ischemic injury area. Third, concomitant diseases (diabetes mellitus, arterial hypertension, vascular dementia, and aging dysfunction of brain metabolism) can significantly decrease the efficiency of a neuroprotector. Fourth, the heterogeneity in location and intensity of cerebral ischemia–reperfusion processes requires the use of different drugs or different doses of a drug. Fifth, it is extremely difficult in clinics to analyze and standardize patients in groups and to select the effect of a drug.4–8

Nanomaterials

The rapid development of nanotechnology has led to the appearance of new prospective engineered nanomaterials for gene and drug therapy, including nanoparticles of metals, quantum dots, fullerenes, carbon nanotubes (CNT), and dendrimers.9 Nanoparticles of metals, mainly iron oxide, gold, and silver, can be used for imaging applications. The optical properties of these nanoparticles can be tuned by changing their size, shape, and surface properties.10 Quantum dots are semiconductor materials in which the absorption and emission spectra are size dependent, so their optical spectrum can be regulated by altering the size of the core. For traditional biological applications, quantum dots have already begun to replace traditional organic fluorophores as simple fluorescent reporters in immunoassays, microarrays, fluorescent imaging applications, and other assay platforms.11 Fullerenes and CNT have a high surface area and internal volume for loading of drugs and imaging agents. They have the potential to deliver drugs directly to targeted cells and tissues. Unfortunately, fullerenes and CNT are not soluble in most organic or aqueous solutions. Surface modification of CNTs is critical for their use in medicine.12 Dendrimers are branching polymers whose structure is formed by monomeric subunit branches diverging to all sides from a central nucleus.13 Low generations of dendrimers have an open, flattened, and asymmetrical shape, but as the generation increases, the structure becomes globular and densely packed at the periphery. The insides of dendrimers are empty cavities. Another important feature of dendrimers is their monodispersity. The classical polymerization process

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From the Republican Research and Practical Center of Neurology and Neurosurgery, Minsk, Belarus (N.S.); Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus, Minsk, Belarus (D.S.); and Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland (M.B.).
Correspondence to Dzmitry Shcharbin, PhD, Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus, Akademicheskaja St 27, Minsk 220072, Belarus. E-mail d.shcharbin@mail.ru
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is usually random and produces molecules of different sizes, whereas the size and molecular mass of a dendrimer can be specifically controlled during synthesis. Finally, dendrimers possess many functional end groups that are responsible for their high solubility and reactivity. Dendrimers have found their place in biology and medicine as follows: contrast agents for MRI, new synthetic vectors for gene therapy, drug-delivery carriers, drugs for treatment of prion diseases and neurodegenerative disorders, and antibacterial and antiviral agents, detoxication agents, antioxidants, and immunostimulating components (adjuvants).14

Nanomaterials in Biology and Medicine
The new unusual properties of nanomaterials are being incorporated into new generations of drug-delivery vehicles, contrast agents, and diagnostic devices. Some of them (nanoparticles of metals, dendrimers, liposomes) are currently undergoing clinical investigation (Phases II or III) or have been approved by the Food and Drug Administration for use in humans.15 Most of them are nanoparticles of metals based on iron oxide or gold for MRI imaging—Feridex, Resovist, Combidex, NanoTherm, Verigene. Only 1 dendrimer-based drug is in the Phase II of clinical trials, the microbicide VivaGel.15 The design considerations for nanomaterials, which are critical for their application in practical medicine, are critical nanoscale design parameters such as: (1) size, (2) shape, (3) surface chemistry, (4) flexibility/rigidity, (5) hydrophobicity, (6) architecture, and (7) elemental composition, that should be controlled to obtain a wide range of synthetic nanostructures. It is especially important when these critical nanoscale design parameters mimic and scale to the dimensions of biological structures or assemblies that influence human health and disease.16,17 Determining and regulating these properties during synthesis, it is possible to provide an exact tuning of nanoparticles both to the concrete disease and to the individual resulting in personal drug design concept.15–17

A simple analysis of publications in the field of nanomaterials according to the SCOPUS database on March 21, 2013, revealed an interesting feature. The total number of publications in the field of nanomaterials is distributed by keywords as follows: nanoparticles, 201,641; CNT, 75,247; quantum dots, 57,523; fullerenes, 29,617; and dendrimers, 16,631 (Table 1). However, although fullerenes, CNT, nanoparticles, and quantum dots have more publications than dendrimers, publications concerning applications of dendrimers in biology and medicine predominate compared with other nanomaterials: dendrimers in biology and medicine, 39% of articles; nanoparticles, 29%; fullerenes, 13%; quantum dots, 11%; and CNT, 9%; analysis was conducted using SCOPUS instruments (Table 1).

Nanomaterials in Stroke Treatment
Recanalization (Thrombolysis)
CNT and Fullerenes
The application of nanomaterials to early recanalization (thrombolysis) in stroke treatment has been studied during the last 5 years. The effect of CNT and fullerenes at this stage is still unknown. However, they can induce blood coagulation.18 Thus, the application of CNT and fullerenes to thrombolysis after stroke requires at least that their surfaces be significantly modified.19

Nanoparticles and Quantum Dots
In contrast, nanoparticles or quantum dots have been used successfully for delivery of tissue plasminogen activator to thrombi to treat acute ischemic stroke. Their advantages are represented by their preferential location within developing thrombi, effectual thrombolysis, and enhanced safety attributable to substantial reduction of the dosage of fibrinolytic agents, and reduced downstream adverse effects.20–22

Dendrimers
Dendrimers are the second prospective candidates, although no data concerning their direct application to stroke treatment have been published. First, they have shown themselves to be effective carriers of heparin,23–25 preventing deep vein thrombosis in a rodent model.26 Second, they are considered to have therapeutic activity against prion diseases.26–28 Cationic dendrimers seem to accumulate together with prion protein in scapie form molecules in lysosomes, where the acidic environment facilitates dendrimer-mediated prion protein in scapie form disaggregation. Superfect, polypropylenimine dendrimers, and phosphorus dendrimers, all proved to have the capacity to remove prion protein in scapie form from cells.26–28 The strength of this effect was dependent on both dendrimer concentration and duration of exposure.30 Third, dendrimers can also be considered potential agents for the disaggregation of amyloid aggregates.31 Different generations of poly(amidoamine) (PAMAM) dendrimers and both polypropylenimine and phosphorus dendrimers have been used successfully to disaggregate amyloid fibrils under different conditions.31–36 Also, PAMAM dendrimers inhibited the fibrillation of α-synuclein, and this effect increased with both generation number and PAMAM concentration.37–39 Among all nanomaterials discussed, dendrimers showed themselves the least toxic.40–42 Dendrimer toxicity in biological system is generally characterized by hemolytic toxicity, cytotoxicity, hemolodic toxicity, and interaction with proteins.40–45 To minimize their toxicity, the critical nanoscale design parameters strategy has been used to synthesize biocompatible dendrimers or to mask peripheral charge of dendrimers by surface engineering. Several types of nontoxic dendrimers have been synthesized.40–43

Neuroprotection
CNT and Fullerenes
The effect of CNT and fullerenes on neuroprotection in stroke treatment is still unknown.

Nanoparticles and Quantum Dots
Nanoparticles or quantum dots have proved themselves to be effective imaging agents without side effects.45,46 Iron oxide nanoparticles showed high efficiency in MR imaging of inflammation in acute brain ischemia,47 whereas ferumoxtran-10 nanoparticles were effective in MR imaging of
been widely used in this direction. The second is den-
terapeutic and diagnostic tools. Further investigations are needed to evaluate inflammation and addi-
tion to erythrocyte deformability. This study suggests that the viscosity of blood plasma. This effect can be attributed to a decrease in hematocrit and hemoglobin concentration in the period of administration: 10 nm nanoparticles decreased the blood–brain barrier. This application is tightly connected to the use of nanoparticles for tracking or targeting of human mesenchymal stem cells for treating stroke. The second possible application of nanoparticles in neuroprotection is targeted delivery of neuroprotective drugs, antioxidant enzymes, or anti-inflammatory agents to the infarction zone. They have been successfully used to deliver the glycineB site antagonist MRZ 2/576 in transient focal ischemia in rats, superoxide dismutase in cerebral ischemia–reperfusion, and tanshinone IIA in cerebral ischemia. An important finding is that liposomes used as model nanoparticles can accumulate in the ischemic core and penumbra region even when cerebral perfusion is reduced. However, the application of nanoparticles was dependent on their size and the period of administration: 10 nm nanoparticles decreased the viscosity of blood plasma. This effect can be attributed to a decrease in hematocrit and hemoglobin concentration in addition to erythrocyte deformability. This study suggests that further investigations are needed to evaluate inflammation and tissue injury in relation to the application of nanoparticles as therapeutic and diagnostic tools.

**Dendrimers**

Applications of dendrimers to neuroprotection in stroke have several facets. The first is the use of dendrimers as coatings for MR agents to improve their redistribution in the body. Starting from the work by Wiener et al., dendrimers have been widely used in this direction. The second is dendrimer-mediated delivery of neuroprotective drugs. N-acetyl cysteine is an anti-inflammatory agent with significant potential for clinical use in the treatment of neuroinflammation, stroke, and cerebral palsy. There is a need to deliver N-acetyl cysteine in ways that enhance its efficacy, reduce dosage, and prevent it from binding plasma proteins. For this purpose, a PAMAM dendrimer–N-acetyl cysteine conjugate was synthesized and evaluated for its release kinetics in the presence of glutathione, cysteine, and bovine serum albumin at both physiological and lysosomal pHs. The conjugate showed an order of magnitude higher antioxidant activity than the free drug and can be used for in vivo studies. Johnson et al. reported the therapeutic potential of S-nitroso-N-acetylcysteine–derivatized generation-4 polyamidoamine dendrimers for reducing ischemia/reper-
fusion injury in an isolated, perfused rat heart. The unique combination of S-nitroso-N-acetylcysteine–derivatized generation-4 polyamidoamine dendrimers dendrimer and a glutathione trigger represents a novel strategy with possible clinical relevance for salvaging ischemic tissue. The third approach, neuroprotection in the postischemic brain by gene therapy using dendrimer-mediated transfection, was proposed by Kim et al. They showed that PAMAM ester dendrimer successfully delivered high-mobility group box 1 small interfering RNA to the rat brain, whereby high-mobility group box 1 expression was depleted in >40% of neurons and astrocytes of the normal brain. Moreover, e-PAM–mediated high-mobility group box 1 small interfering RNA delivery notably reduced infarct volume in the postischemic rat brain, generated by occluding the middle cerebral artery for 60 minutes. These results indicate that e-PAM–R, a novel biodegradable nonviral gene carrier, offers an efficient means of transfecting small interfering RNA into primary neuronal cells in the brain and of performing small interfering RNA–mediated gene knockdown. The data of Kim et al. are supported by results on efficient dendrimer–mediated delivery into the brain through the blood–brain barrier. Dendrimers can deliver genetic material, peptides, or the anticancer drug paclitaxel to the brain. Moreover, PAMAM dendrimers can restore blood–brain barrier permeability in rats with diabetes mellitus. This opens prospects for reducing blood–brain barrier dysfunction in stroke using these nanomaterials.

Summarizing, the prospects for using CNT and fullerenes in stroke treatment are still unknown. In contrast, new developments...
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siRNAs indicates small interfering RNAs.

in medical applications of nanoparticles and dendrimers related to cerebral ischemia make them good candidates for nanomaterial-driven stroke treatment (Table 2). Systematic studies concerning nanomaterials (efficiency and toxicity)\(^7\) are required for the development of new nanomaterial-based drugs.

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None.

References


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