Highly bioactive zeolitic imidazolate framework-8–capped nanotherapeutics for efficient reversal of reperfusion-induced injury in ischemic stroke

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Rational design of potent antioxidative agent with high biocompatibility is urgently needed to treat ischemic reperfusion-induced ROS-mediated cerebrovascular and neural injury during ischemia strokes. Here, we demonstrate an in situ synthetic strategy of bioactive zeolitic imidazolate framework-8–capped ceria nanoparticles (CeO2@ZIF-8 NPs) to achieve enhanced catalytic and antioxidative activities and improved stroke therapeutic efficacy. This nanosystem exhibits prolonged blood circulation time, reduced clearance rate, improved BBB penetration ability, and enhanced brain accumulation, where it effectively inhibits the lipid peroxidation in brain tissues in middle cerebral artery occlusion mice and reduces the oxidative damage and apoptosis of neurons in brain tissue. CeO2@ZIF-8 also suppresses inflammation- and immune response–induced injury by suppressing the activation of astrocytes and secretion of proinflammatory cytokines, thus achieving satisfactory prevention and treatment in neuroprotective therapy. This study also sheds light on the neuroprotective action mechanisms of ZIF-8–capped nanomedicine against reperfusion-induced injury in ischemic stroke.

INTRODUCTION

Ischemic stroke, accounting for about 85% of strokes and having increasing mortality and long-term disability rates, is one of the most serious public health problems (1, 2). In the process of reperfusion, blood flow restoration in the ischemic brain not only improves the oxygen supply but also induces the overproduction of reactive oxygen species (ROS), including superoxide anion (·O2−), hydrogen peroxide (H2O2), and hydroxyl radical (·OH), to cause the secondary injury in cerebrovascular system and neural networks (3, 4). The excessive ROS generation can mediate inflammation and immune response by stimulating the expression of cytokines and adhesion molecules, ultimately causing further injury by inflammation, leading to ischemia–reperfusion injury (5, 6). Edaravone-based neuroprotective therapy is the main strategy for the treatment of ischemic stroke by eliminating the free radicals and suppressing oxidative stress (7–9). However, its clinical application is limited by the low bioavailability, short half-life, inefficient penetration across the blood–brain barrier (BBB), and the side effects to kidney and liver functions. Therefore, the design and development of agents with potent ROS scavenging activity and desirable physicochemical property for the treatment of ischemia strokes is urgently needed.

Nanotechnology-mediated antioxidative therapy has been proven as a promising method for the treatment of the diseases induced by oxidative damage (10–13). Until now, various nanoantioxidants, such as melanin (14), carbon, platinum, ceria (CeO2), manganese (15), and magnetite/ceria nanoparticle (NP) (16), have been designed to achieve efficient treatments for stroke and other chronic diseases. Different nanosystems have been synthesized to mediate intracellular ROS to achieve therapeutic use (17, 18). Among these nanomaterials, CeO2 NPs have attracted great interest owing to their high antioxidant activity and recyclable ROS scavenging ability (19). Because of the fluorite lattice structure, under physiological conditions, CeO2 NPs could easily lose oxygen or get electrons, resulting in the formation of oxygen vacancy and lowered valence states, and the electron transfer between Ce(III) and Ce(IV) endows it with potent antioxidant activity and repetitive ROS elimination ability (20). For instance, Kim et al. (21) have reported that ceria NPs can protect brain tissue against oxidative damage during ischemic stroke. Bao et al. (22) have designed and synthesized effective stroke treatment agents based on monodisperse CeO2 NPs with simultaneous BBB crossing and protection activity by loading with edaravone. In addition, neutrophil and red blood cell membranes were also used to decorate nanovesicles for ischemic stroke therapy (23, 24). However, the further clinical development of these stroke treatment NPs is limited by the short vascular circulation time, easy interparticle aggregation, and undesirable occurrence of direct catalytic reaction on exposed active sites. Therefore, the construction of a stable and biocompatible shell encapsulation of the chemically active CeO2 NPs is a good way to overcome these drawbacks.

As unique porous materials, metal–organic frameworks (MOFs) have been recognized as potential encapsulation shell and drug carriers for biomedical applications due to their high porosity, large surface areas, tunable functionality, and well-defined pore structures (25, 26). Zeolitic imidazolate framework–8 (ZIF-8) is one of the most promising representatives of MOFs built from zinc ions and 2-methylimidazolate (2-MI), thus exhibiting nontoxic and biocompatible advantages (27). Nanoscale ZIF-8 was stable under physiological conditions and could decompose under acidic environments because of protonation effect (28), such as tumor tissues and acidic organelles, thus making it highly suitable for constructing pH-responsive drug delivery systems. ZIF materials have also been applied in encapsulation of small-molecule drugs, larger drug molecules, enzymes, and even NPs, including numerous noble metal NPs such as Pt, Pd, and Au, to develop core/shell nanocomposites with new functions and biological applications (25, 29–31). The encapsulation with ZIF could modify the surface properties of NPs and prevent the aggregation, thus enhancing the stability under physiological conditions. Moreover, the cavity and porous structure could endow the materials with size-sieving behavior to regulate the diffusion and reaction process.
of large molecules to increase reaction selectivity (25). Inspired by this, it is possible that the unique porous and bioresponsive properties of ZIF-8 can help to protect the exposed active sites in CeO2 NPs and to regulate the controlled release of active components.

Therefore, in this study, we explored a new in situ synthesis strategy of ZIF-8–capped CeO2 NPs (CeO2@ZIF-8) with enhanced catalytic and antioxidative activities. This smart design can overcome the drawbacks of CeO2 NPs and achieve the following advantages: (i) The surface ZIF-8 acts as peroxidase to maintain the antioxidative activity in the presence of excessive H2O2 or other oxidants, which can absorb H2O2 and destroy the O–O bond to disintegrate H2O2. (ii) The ZIF-8 frame growing on the outer layer of CeO2 NPs controls the size, shape, and surface charge of CeO2 core to make it more suitable for biological application. (iii) The decomposition of ZIF-8 results in the release of active components, which synergistically enhance the stroke therapeutic efficacy of CeO2. As expected, CeO2@ZIF-8 NPs exhibit effective ROS scavenging in vitro and protect PC12 neuronal cells from free radical–induced apoptosis. ZIF-8 encapsulation can also effectively prolong the blood circulation time of CeO2, reduce the clearance rate, improve the penetration across BBB, and enhance its accumulation in brain tissue. As a result, this nanosystem effectively inhibits the lipid peroxidation in brain tissues of middle cerebral artery occlusion (MCAO) model mice, reduces the oxidative damage and apoptosis of neurons in brain tissue, and suppresses the inflammation–immune response–induced injury, thus achieving satisfactory prevention and treatment in neuroprotective therapy during ischemic stroke with high safety. Together, this study not only provides a new in situ synthetic approach of synergistic nanotheapeutics by using ZIF as bioactive surface decoration and CeO2 NPs as functional core but also sheds light on the neuroprotective application mechanisms against reperfusion-induced injury in ischemic stroke.

RESULTS AND DISCUSSION

Rational design and in situ synthesis of ZIF-capped CeO2 NPs

In this study, we have rationally designed and synthesized ZIF-8–capped CeO2 NPs (CeO2@ZIF-8 NPs; Fig. 1A) to achieve enhanced catalytic and antioxidative activities. In this nanosystem, ZIF-8 encapsulation can control size and surface charge of CeO2 core to make it more suitable for biological application. First, CeO2 nanopolyhedra were successfully created through a facile hydrothermal method with a size of ~20 ± 5 nm (fig. S1, A and B), ZIF-8 with a nanoscale at ~140 nm (Fig. 1B) was synthesized according to previous procedures with modification. Here, we also explored a new in situ synthesis method of CeO2@ZIF-8 composite nanomaterials, which make the ZIF-8 frame grow on the outer layer of CeO2. The synthesized CeO2@ZIF-8 nanomaterials appear as highly monodisperse particles with a diameter of ~240 nm (Fig. 1C), and CeO2 is distributed within the framework of ZIF-8, as shown in the elemental mapping of Ce and Zn (Fig. 1, D and E). As examined by a laser particle size and zeta potential analyzer, the average particle sizes of CeO2, ZIF-8, and CeO2@ZIF-8 are ~88.3, ~168, and ~275 nm, respectively, and the zeta potentials are ~23.4, ~4.9, and ~10.5 eV, respectively (fig. S1, C and D). Furthermore, CeO2@ZIF-8 exhibited the highest stability in aqueous solution and Dulbecco’s modified Eagle’s medium (DMEM) (fig. S1E). We also used transmission electron microscopy (TEM) to investigate the morphology of CeO2@ZIF-8 in H2O2, DMEM, and phosphate-buffered saline (PBS) for 24 and 48 hours. As shown in fig. S1F, CeO2@ZIF-8 was slightly degraded in H2O2 for 24 and 48 hours. However, it was unstable in the PBS (pH 7.4) buffer and could slowly decompose during the incubation. In contrast, in DMEM with 10% fetal bovine serum (FBS), CeO2@ZIF-8 was kept stable even after 48-hour incubation, which could be due to the formation of protein corona that could protect the nanosystem during blood circulation. X-ray diffraction (XRD) pattern showed that, after growth in the framework of ZIF-8, CeO2 still has a pure and typical fluorite cubic structure, and the outer layer of ZIF-8 retains its crystal structure (Fig. 1F). Furthermore, CeO2@ZIF-8 exhibits the same characteristic absorption peak with pure CeO2 in ultraviolet-visible (UV-vis) spectra, indicating the presence of CeO2 in ZIF-8 (Fig. 1G). Raman spectra of CeO2@ZIF-8 show the characteristic absorption peaks of CeO2 and ZIF-8, and no new absorption peaks are formed, which indicates that there is no new chemical bond between CeO2 and ZIF-8 (Fig. 1H). As shown in Fig. 1I, the presence of Ce 3d and Zn 2p peaks in the x-ray photoelectron spectroscopy (XPS) spectrum of CeO2@ZIF-8 further confirmed the successful synthesized composite nanomaterial with CeO2 and ZIF-8. The unaltered Zn 2p and Ce 3d spectra of ZIF-8, CeO2, and CeO2@ZIF-8 further confirmed that no new chemical bonds were formed between ZIF-8 and CeO2 (Fig. 1, J and K). Meanwhile, the content ratio of CeO2 in CeO2@ZIF-8 composite nanomaterials was ~0.278 mg CeO2/mg CeO2@ZIF-8 by inductively coupled plasma mass spectrometry (ICP-MS) analysis. These results confirm the successful synthesis of CeO2@ZIF-8 NPs.

Enhancement of ROS scavenging by ZIF encapsulation

It is well known that ROS are excessively produced during ischemia-reperfusion in stroke, resulting in damage to the cerebrovascular system and neural networks (32, 33). CeO2 NPs have been reported as effective free radical scavengers to prevent neuron damage by ROS (15, 19). In this study, we have evaluated the ROS scavenging activity of CeO2, ZIF-8, and CeO2@ZIF-8 (Fig. 2A). First, we examined the total antioxidant activities of CeO2, ZIF-8, and CeO2@ZIF-8 by ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] free radical scavenging assays. As shown in Fig. 2B and Fig. S2 (A to C), CeO2@ZIF-8 notably inhibits the formation of ABTS free radicals in a time- and dose-dependent manner, suggesting higher antioxidant activity than those of ZIF-8 and CeO2 nanopolyhedra alone. We then examined the reactions of CeO2, ZIF-8, and CeO2@ZIF-8 with H2O2 by in situ Raman spectroscopic. As shown in Fig. 2C and Fig. S2 (D and E), after adding H2O2 onto the surfaces of CeO2, ZIF-8, and CeO2@ZIF-8 nanomaterials, the peak of 880 cm−1 immediately appeared, and the special peaks of ZIF-8 and CeO2 disappeared in the Raman spectra of these nanomaterials. The new peak formation may be due to the formation of O=O stretching after adsorption of O2− from H2O2. Furthermore, as the reaction progresses, the peak of 880 cm−1 weakens or even disappears, and the characteristic peak of CeO2 at 460 cm−1 reappears after 40 min, while the special peaks of ZIF-8 did not reappear after reaction with H2O2. We then examined the morphological changes of ZIF-8 after reaction with H2O2 by TEM. As shown in Fig. 2D, after incubation with 5% H2O2 for 3 hours, the ZIF-8 NPs were broken into pieces, and CeO2@ZIF-8 was cracked to leave CeO2 nanopolyhedra, indicating that the ZIF-8 NPs can be degraded in the environment of 5% H2O2. These results indicate that CeO2 and CeO2@ZIF-8 have excellent recyclable antioxidant property to react with H2O2 repeatedly due to the presence of CeO2 nanopolyhedra.
Furthermore, electron paramagnetic resonance (EPR) spectra were used to examine the $^\cdot$OH scavenging by CeO$_2$, ZIF-8, and CeO$_2$@ZIF-8 nanomaterials. The $^\cdot$OH is generated through the Fenton reaction with Fe$^{2+}$/H$_2$O$_2$ system and detected by 5,5′-dimethylpyrrolidine-1-oxide (DMPO). As shown in Fig. 2E, the EPR spectra of Fenton reaction induce the special signals of DMPO-OH adducts, suggesting the successful generation of $^\cdot$OH.

After adding CeO$_2$, ZIF-8, and CeO$_2$@ZIF-8 in Fe$^{2+}$/H$_2$O$_2$ system, the signal intensity sharply decreased, especially for CeO$_2$@ZIF-8, with the same concentration of 15 $\mu$g/ml. We then also examined the $^\cdot$OH scavenging activity by...
UV-vis spectroscopy. As shown in Fig. 2F, UV-vis spectrum shows a special peak at 520 nm due to the reaction of salicylic acid (SA) with •OH generated by the Fenton reaction. As expected, CeO₂@ZIF-8 effectively scavenged the •OH free radical, as demonstrated by the decrease in absorbance at 520 nm and changes in color of the mixed solution. We also observed the strong antioxidant activity of the ligand 2-MI, while no free radical scavenging effect was observed for the Zn²⁺ ion (fig. S2, F and G), indicating that the antioxidant activity of ZIF-8 is attributed to the 2-MI ligand.

We then also conducted the EPR spectra to examine the "O₂⁻" scavenging ability of CeO₂, ZIF-8, and CeO₂@ZIF-8. "O₂⁻" was generated by the reaction of xanthine and xanthine oxidase and detected by the dihydroethidium (DHE) probe. As shown in Fig. 2G, all three NPs reduced the EPR amplitude of DMPO-OOH, especially the CeO₂@ZIF-8 composite nanomaterials. Furthermore, we also examined the "O₂⁻" scavenging ability of CeO₂@ZIF-8 using DHE probe by fluorescence spectra. As shown in Fig. 2H, CeO₂@ZIF-8 decreased the intensity of the special peak in a dose-dependent manner, which was much higher than those of CeO₂ NPs and ZIF-8 alone. The formation of CeO₂@ZIF-8 notably decreased the surface area, pore volume, and pore size of ZIF-8 (fig. S3). Such a more conservative shell encapsulation could help prevent the direct catalytic reaction on exposed CeO₂ active sites, thus achieving better long-term and controllable effects.

**Protection of pheochromocytoma cells by CeO₂@ZIF-8 against oxidative stress–induced damage**

The rat adrenal medulla pheochromocytoma PC12 cell line has general characteristics of neuroendocrine cells, which is widely used in the study of neurophysiology and neuropharmacology. Therefore, we used PC12 cells damaged by ROS oxidation as a cell model for ischemic injury in stroke and further examined the protection of CeO₂@ZIF-8 to PC12 cells against tert-butyl hydroperoxide (t-BOOH)–induced oxidative damage. First, we detected the cytotoxic effects of CeO₂, ZIF-8, and CeO₂@ZIF-8 nanomaterials against PC12 cells after 48-hour incubation. As shown in fig. S4A, none of the three NPs
exhibited cytotoxicity to PC12 cells. As illustrated in Fig. 3A, 20 μM t-BOOH significantly inhibited PC12 cell proliferation and induced the cells’ viability at 55.6%. CeO₂, ZIF-8, and CeO₂@ZIF-8 nanomaterials reversed cell damage induced by t-BOOH, especially CeO₂@ZIF-8. Furthermore, CeO₂@ZIF-8 protects PC12 cells from t-BOOH–induced oxidative damage in a dose-dependent manner (Fig. S4B). Apoptosis is the main action mode of t-BOOH to cause cell growth inhibition. Therefore, we performed the annexin V and propidium iodide (PI) containing assay to examine the reversal of t-BOOH–induced apoptosis by CeO₂@ZIF-8. As shown in Fig. 3B, t-BOOH notably enhanced PC12 cell apoptosis, especially in late stage, while CeO₂@ZIF-8 effectively reduced the cell apoptosis in a dose-dependent manner. For instance, 20 μM t-BOOH induced cell apoptosis at late stage with 57.2%, while after being cotreated with CeO₂@ZIF-8 (4 μg/ml), the cell apoptosis at late stage decreased 9.1%. Then, we further used flow cytometry to examine the apoptotic cell death and cell cycle distribution of PC12 cells exposed to t-BOOH and CeO₂@ZIF-8. As shown in Fig. 3C and fig. S4C, CeO₂@ZIF-8 could reduce t-BOOH–induced PC12 cell apoptosis and cell cycle arrest. CeO₂@ZIF-8 protecting PC12 cells from t-BOOH–induced oxidative damage may be due to its efficient free radical scavenging ability. Excess intracellular ROS cause DNA damage and induce cell apoptosis. Therefore, we further tested the ability of CeO₂@ZIF-8 to scavenge intracellular ROS by DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate) assay. As shown in Fig. 3D, t-BOOH notably promoted ROS overproduction in PC12 cell to 140% in the first 18 min, and the ROS level remained at 120% even at 120 min. However, after incubation with CeO₂@ZIF-8, ROS generation in t-BOOH–treated PC12 cells was notably reduced. For instance, CeO₂@ZIF-8 (4 μg/ml) rapidly scavenged intracellular ROS generation to 105% in the first 18 min and further reduced the ROS level to the initial state of PC12 cells by 66 min. Furthermore, we also used fluorescence imaging to examine the fluorescence intensity of ROS levels in t-BOOH–damaged PC12 cells after treatment with CeO₂@ZIF-8. As shown in fig. S4D, t-BOOH triggered the ROS overproduction in PC12 cells, as reflected by the notable increase in green fluorescence. CeO₂@ZIF-8 notably reduced the fluorescence intensity in PC12 cells in a.
dose-dependent manner, indicating the effective ROS scavenging in cell model by CeO$_2$@ZIF-8.

Mitochondria are major organelles for energy generation and the main source of generation of ROS, which play an important role in regulating the cell function and fate (34). If the excess superoxide is not cleared by intracellular antioxidants or related enzymes, it will lead to oxidative stress damage and dysfunction of mitochondria, resulting in cell death and other lesions. As illustrated in Fig. 3E, the mitochondria in healthy cells exhibit the filamentous mitochondrial network, while after incubation with 15 μM t-BOOH, the mitochondria were obviously destroyed severe fragments. Moreover, CeO$_2$@ZIF-8 effectively inhibited the mitochondrial fragmentation by t-BOOH. The morphological improvements further indicated that CeO$_2$@ZIF-8 could protect PC12 cells from t-BOOH--induced mitochondrial fragmentation through scavenging intracellular excess ROS.

Enhancement of transportation across the BBB of CeO$_2$@ZIF-8 and the endocytosis by PC12 cells

The capacity to penetrate across BBB is an essential factor for drug delivery to cure ischemic cerebral stroke. Although BBB can be disrupted by excess ROS during ischemia-reperfusion in stroke, studies also found that the damaged BBB in opening state lasts for only several hours (35, 36). Therefore, the permeability of nanodrugs across the BBB to enhance its accumulation in the brain lesion is essential for stroke therapy. In this study, we conducted transwell assay for the coculture of human brain microvascular endothelial cell (HBMEC)/PC12 cell to simulate the BBB model and evaluated the permeability of coumarin 6 (C6)--labeled CeO$_2$@ZIF-8 (Fig. 3F). As shown in Fig. 3G, CeO$_2$@ZIF-8 effectively transports across the HBMECs after 24-hour incubation, and the transport ratios of CeO$_2$@ZIF-8 NPs at 4 μg/ml reached 37.1%. In addition, we also examined the internalization of the penetrative CeO$_2$@ZIF-8 in the lower chamber of PC12 cells by fluorescence imaging. As shown in Fig. 3H, the fluorescence intensities of C6-labeled CeO$_2$@ZIF-8 in PC12 cells were enhanced in a dose-dependent manner, which suggested the high permeability of CeO$_2$@ZIF-8 through the BBB.

Lysosome-mediated endocytosis is an important mechanism for nanomaterials to enter cells (37). In this study, we examined the localization of CeO$_2$@ZIF-8 in PC12 cells by TEM and fluorescence imaging and proposed a cell internalization mechanism (Fig. 4A). As shown in Fig. 4B and fig. S5A, CeO$_2$ nanopolyhedra, but not CeO$_2$@ZIF-8, were found in the lysosome of the cell. This may be due to the degradation of the outer framework of ZIF-8 in the lysosomal environment. Therefore, we further examined the morphological changes of CeO$_2$@ZIF-8 after incubation in different pH of PBS solutions for 12 hours by TEM. As shown in Fig. 4C, after being shaken for 12 hours in PBS at pH 7.4 (simulating the physiological condition), CeO$_2$@ZIF-8 still maintained the same morphology as that in aqueous solution. After incubation in PBS at pH 5.3 with lysozyme (simulating the acidic lysosomal environment), the outer framework of ZIF-8 was cracked, leaving CeO$_2$ nanopolyhedra, indicating that the ZIF-8 NPs can be degraded in the lysosomal environment. To further explore this hypothesis, we then synthesized the C6-labeled CeO$_2$@ZIF-8 to explore its intracellular trafficking by fluorescence imaging. First, PC12 cells were stained with special fluorescent tracers, LysoTracker (red) and Hoechst 33342 (blue). Figure 4D shows that C6-labeled CeO$_2$@ZIF-8 (green fluorescence) gathered in the cell membrane after 0.5-hour treatment and started to enter in lysosomes at 1 hour. Furthermore, the green fluorescence of C6-labeled CeO$_2$@ZIF-8 matches well with the red fluorescence from LysoTracker after 2-hour incubation, and the green fluorescence intensity enhanced gradually thereafter. These images suggest that CeO$_2$@ZIF-8 internalizes in PC12 cells through lysosome-mediated endocytosis.

To further explore the internalization pathway of CeO$_2$@ZIF-8 in PC12 cells, we examined the cellular uptake behavior after being performed with different chemical endocytosis inhibitors. First, we examined the quantitative cellular uptake of CeO$_2$@ZIF-8 in PC12 cells, finding that the intracellular concentration of nanomaterials increased in a time- and dose-dependent manner in PC12 cells (Fig. 4E). We then explored the cellular uptake of CeO$_2$@ZIF-8 in PC12 cells after pretreatment with different endocytosis inhibitors. As shown in Fig. 4F, these endocytosis inhibitors significantly inhibit the internalization of CeO$_2$@ZIF-8 in PC12 cells, especially the nystatin, suggesting that lipid raft–dependent endocytosis was the main pathway. Furthermore, sodium azide (NaN$_3$), chlorpromazine (CPZ), and low temperature (4°C) are the inhibitors of energy-dependent endocytosis. From the results, we found that these effectively inhibited the cellular uptake of CeO$_2$@ZIF-8, especially the combined treatment of NaN$_3$ and CPZ with low temperature, respectively. Sucrose is a specific inhibitor of clathrin-mediated endocytosis, and dynasore acts as an inhibitor of dynamin-mediated lipid raft endocytosis. After cotreatment with 4°C, these two inhibitors further decreased the internalization of CeO$_2$@ZIF-8 in PC12 cells (Fig. 4G). Together, both energy- and lipid raft–dependent endocytosis were the main pathways of CeO$_2$@ZIF-8 being internalized in PC12 cells.

In vivo antagonism of ischemic stroke by CeO$_2$@ZIF-8

To further confirm the in vivo brain protection of CeO$_2$@ZIF-8, we established the MCAO rat model to simulate the generating process of ischemia-reperfusion in stroke. The MCAO model was formed by inserting the filament with a silicone tip into the middle cerebral artery of the rat for 90 min, and then the filament was removed to form the reperfusion. The infarct areas and neurological scores of MCAO mice were analyzed after treatment with CeO$_2$@ZIF-8 for 3 days. As shown in Fig. 5 (A and B), the MCAO model mice with saline injection only showed large-area infarction, which is reflected by the inability stained by 2,3,5-triphenyltetrazolium chloride (TTC), while after treatment with CeO$_2$@ZIF-8 for 3 days in MCAO mice, the brain infarct area significantly decreased in a dose-dependent manner. For instance, a large infarct area with ~42.3% was detected in the saline group, while after injection with CeO$_2$@ZIF-8 at 0.2 and 0.4 mg/kg, the infarct area decreased to 23.1 and 15.7%, respectively. Furthermore, the neurological scores of MCAO mice after treatment with CeO$_2$@ZIF-8 also improved (Fig. 5C). The body weight of MCAO mice showed little change in each treatment group (fig. S5B), indicating the high in vivo anti–ischemic stroke activity and low toxicity of CeO$_2$@ZIF-8 nanosystem. To further explore the enhancement of brain protection of CeO$_2$@ZIF-8, we also constructed C57 mice MCAO model to examine the effects of different nanomaterials on the infarct areas and neurological scores after treatment for 3 days. As shown in fig. S6 (A and B), CeO$_2$ NPs, ZIF-8, and CeO$_2$@ZIF-8 significantly decreased the brain infarct area, especially CeO$_2$@ZIF-8. We also conducted the Bederson’s scoring system and elevated body swing test to evaluate the effects of these NPs on the function and behavior of the MCAO mice. The results showed that treatments with CeO$_2$, ZIF-8, and CeO$_2$@ZIF-8 effectively improved the function and behavior of MCAO mice, with better effects found in the CeO$_2$@ZIF-8 group (fig. S6, C to E).
To further investigate the mechanism of the anti–ischemic stroke activity, we first examined the internalization of CeO$_2$@ZIF-8 in the brain tissue of MCAO mice using in vivo fluorescence imaging and TEM observation. As shown in fig. S7A, indocyanine green (ICG)–labeled CeO$_2$@ZIF-8 can quickly accumulate into brain tissue after intravenous injection, indicating that this nanosystem can cross BBB along with the restored perfusion in MCAO mice. Furthermore, from the TEM image of brain tissue, CeO$_2$ nanopolyhedra were found in the brain tissue of MCAO mice after intravenous injection three times with CeO$_2$@ZIF-8 for 3 days (Fig. 5D and fig. S7B). The results indicated that CeO$_2$@ZIF-8 can penetrate BBB to reach brain tissue and break down the outer ZIF-8 to release a large amount of CeO$_2$ nanopolyhedra. Therefore, we then further examined the biodistribution of Ce in the main organs of mice after intravenous injection with CeO$_2$ and CeO$_2$@ZIF-8. As shown in Fig. 5E, compared with the treatment group of CeO$_2$, the content of Ce in brain tissue was significantly enhanced and decreased in the liver tissue after injection with CeO$_2$@ZIF-8, suggesting that CeO$_2$@ZIF-8 composite nanomaterials decrease the clearance of the free CeO$_2$ by murine macrophages in liver and thus enhance their accumulation in brain tissues. Further studies were conducted to explore the metabolism of CeO$_2$@ZIF-8 in the main organs for a long time. As shown in fig. S7C, we found that the accumulation of Ce in liver, spleen, and kidney significantly decreased after intravenous injection with CeO$_2$@ZIF-8 for 7 and 14 days, indicating that CeO$_2$@ZIF-8 can effectively be cleared out in vivo. To further verify this hypothesis, we then investigated the pharmacokinetic property of CeO$_2$ and CeO$_2$@ZIF-8 in SD mice. First, the concentration of Ce in plasma after injection of CeO$_2$@ZIF-8 was much higher than that of the free CeO$_2$ group (Fig. 5F). Furthermore, from the related pharmacokinetic parameters of these two nanomaterials, the elimination rate (k$_{10}$, k$_{12}$, and k$_{21}$) and clearance of CeO$_2$@ZIF-8 significantly decreased compared with the free CeO$_2$ nanopolyhedra after loading into the framework of ZIF-8 (table S1). The half-life (t$_{1/2}$ alpha and t$_{1/2}$ beta) and mean retention time of CeO$_2$@ZIF-8 were much higher than those of the free CeO$_2$ nanopolyhedra. These results showed that

![Fig. 4. Endocytosis of CeO$_2$@ZIF-8 in PC12 cells. (A) Schematic demonstration of endocytosis of CeO$_2$@ZIF-8 in PC12 cells. (B) TEM image of CeO$_2$@ZIF-8 internalized in PC12 cells. PC12 cells were incubated with CeO$_2$@ZIF-8 (16 µg/ml) for 6 hours. (C) TEM images of CeO$_2$@ZIF-8 in aqueous (I), PBS (pH 7.4; II), and PBS (pH 5.3 with lysozyme; III) after shaking for 12 hours. (D) Intracellular trafficking of C6-labeled CeO$_2$@ZIF-8 (20 µg/ml; green fluorescence) in PC12 cells. The cells were stained with special fluorescent tracers: LysoTracker (red) and Hoechst 33342 (blue). (E) Quantitative analysis of cellular uptake of CeO$_2$@ZIF-8 in PC12 cells by determination of fluorescence intensity. (F and G) Intracellular uptake inhibition by different endocytosis inhibitors. The cells were pretreated with different endocytosis inhibitors for 1 hour and then incubated with CeO$_2$@ZIF-8 (10 µg/ml) for 2 hours at 37° or 4°C. The control group was incubated with CeO$_2$@ZIF-8 at 37°C only. **P < 0.01 and ***P < 0.001.](http://advances.sciencemag.org/)
loading CeO₂ into ZIF-8 could effectively prolong the blood circulation time of CeO₂, reduce the clearance rate, and enhance the accumulation in brain tissue.

On the basis of the efficient ROS scavenging ability of CeO₂@ZIF-8 in vitro, we then further explored the mechanism of the anti-ischemic stroke activity through scavenging the ROS in vivo. As shown in Fig. 5G, the superoxide anion in the brain tissue of the MCAO model mice with saline injection only was significantly increased compared with the sham-operated control group. However, after injection with CeO₂@ZIF-8 for 3 days in MCAO mice, the superoxide anion level decreased in a dose-dependent manner. Malondialdehyde (MDA) is one of the most important products of membrane lipid peroxidation, which affects the activities of respiratory chain complexes and key enzymes in mitochondria. Therefore, we also examined the production of MDA in the brain tissue of the MCAO mice and found that the MDA level in the brain tissue of the MCAO mice after treatment with CeO₂@ZIF-8 was much lower than that in the MCAO mice with saline injection only (Fig. 5H). Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are both important antioxidative enzymes in the human body, which exhibit efficient ROS scavenging ability to repair cells and reduce the damage by ROS. In this study, we then further examined the SOD and GSH-Px contents in brain tissues of MCAO mice. As shown in Fig. 5I, the SOD content in brain tissue of the MCAO mice was much higher than that in the sham-operated control group, indicating that oxidative stress causes the up-regulation of SOD, while after injection with CeO₂@ZIF-8 at 0.2 and 0.4 mg/kg three times, the SOD content in the brain tissue of these treated MCAO mice did not increase further. Even in the brain tissue of these MCAO mice, the GSH-Px content was decreased compared with control group (Fig. 5J). These results indicate that CeO₂@ZIF-8 inhibits lipid peroxidation in brain tissues of MCAO mice by direct scavenge of ROS overproduction, but not up-regulation of enzymatic activities of SOD and GSH-Px.

Furthermore, we also elucidated the therapeutic effects of CeO₂@ZIF-8 as a neuroprotective agent by pathological analysis in brain tissue. First, from the results of hematoxylin and eosin (H&E) staining in brain sections of MCAO mice, the group of saline injection only showed massive necrosis in the cerebral infarction area, while...
CeO$_2$@ZIF-8 notably decreased the area of necrosis (Fig. 6A). Therefore, we also examined the damage to neurons in the infarcted area of MCAO mice using Nissl staining. As shown in Fig. 6B, compared with the sham-operated control group, the number of intact neurons was notably decreased and exhibited irregular morphology and disordered arrangement in the infarcted area of MCAO mice with saline injection only. However, after treatment with CeO$_2$@ZIF-8 for 3 days in MCAO mice, the neuron damage in brain tissue decreased in a dose-dependent manner. In addition, from the result of TUNEL (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling)–Hoechst costaining assay in brain sections of MCAO mice, the number of intact neurons was notably decreased and exhibited irregular morphology and disordered arrangement in the infarcted area of MCAO mice with saline injection only. However, after treatment with CeO$_2$@ZIF-8 for 3 days in MCAO mice, the neuron damage in brain tissue decreased in a dose-dependent manner. In addition, from the result of TUNEL (terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling)–Hoechst costaining assay in brain sections of MCAO mice, the group of saline injection exhibited typical apoptotic characteristics in the infarcted area, while CeO$_2$@ZIF-8 notably decreased the apoptosis of neurons in a dose-dependent manner (Fig. 6C). These results further indicate that CeO$_2$@ZIF-8 effectively inhibits the damage and apoptosis of neurons, thus reducing the further oxidative damage caused by ischemia-reperfusion in stroke.

**CeO$_2$@ZIF-8 suppresses inflammation and immune response induced by reperfusion in ischemic stroke**

A large number of glial cells proliferate and activate during ischemia-reperfusion injury, resulting in strong inflammatory reaction and glial scar formation, ultimately causing the further injury by inflammation (28, 33). Among them, astrocytes and microglia are the main inflammatory cells in brain tissue, and their activation reflects the state of inflammation in the brain. Therefore, we have examined the expression level of glial fibrillary acidic protein (GFAP; a marker of astrocytes) and ionized calcium–binding molecule-1 (Iba-1; a marker of microglia) in brain sections of MCAO mice by immunohistochemical staining. As shown in Fig. 6D, compared with the sham-operated control group, the expression levels of GFAP and Iba-1 were much higher in brain sections of MCAO mice with saline injection only, indicating that MCAO model induces enhancement in the number of astrocytes and microglia. After treatment with CeO$_2$@ZIF-8 for 3 days in MCAO mice, the number of GFAP- and Iba-1–positive cells was significantly decreased in a dose-dependent manner (Fig. 6, E and F). We also examined the proinflammatory cytokines of tumor necrosis factor–α (TNF-α) and various kinds of interleukins, such as IL-1β and IL-6 secreted in the infarct part of the brain tissue of the MCAO mice. As shown in Fig. 6 (G to I), MCAO model induced significant enhancement secretion of TNF-α, IL-1β, and IL-6 in brain tissue compared with the sham-operated control group. After treatment with CeO$_2$@ZIF-8 for 3 days in MCAO mice, the levels of these proinflammatory cytokines showed...
The surface ZIF-8 acts as peroxidase to maintain the antioxidant protective application mechanisms against reperfusion-induced injury, thus reducing further injury by inflammation.

Toxic side effects of nanomedicine on human body are an important limitation for their future biomedical application. Therefore, we further performed the histological analysis in the main organs by H&E staining in these MCAO mice. As shown in fig. S8, the sections of major organs, including heart, liver, spleen, lung, and kidney, did not exhibit the obvious inflammation or other pathological changes after treatment with CeO$_2$ @ZIF-8 for 3 days in MCAO mice. Furthermore, we also examined the in vivo toxicity of CeO$_2$ @ZIF-8 in healthy mice for a long time. The hematological and pathological analysis reveals that, after 14-day treatment, CeO$_2$ @ZIF-8 demonstrated no obvious damage to these major organs of the mice under this experimental condition (fig. S9), indicating the safety potency of this nanosystem in future application. Together, this study demonstrates an effective and safe ROS scavenging agent to protect the ischemia-reperfusion injury during stroke in vivo.

Currently, for clinical use purposes, it is urgently needed to design and develop agents with potent antioxidative activities and desirable physicochemical property for the treatment of ischemia strokes. Therefore, in this study, we have rationally designed and synthesized ZIF-capped CeO$_2$ NPs (CeO$_2$ @ZIF-8 NPs) with enhanced catalytic and antioxidative activities. This smart design could overcome the drawbacks of CeO$_2$ NPs and achieve the following advantages: (i) The surface ZIF-8 acts as peroxidase to maintain the antioxidant activity in the presence of excessive H$_2$O$_2$ or other oxidants, which can absorb H$_2$O$_2$ and destroy the O=O bond to disintegrate H$_2$O$_2$. (ii) The ZIF-8 frame growing on the outer layer of CeO$_2$ NPs controls the size, shape, and surface charge of CeO$_2$ core to make it more suitable for biological application. (iii) The decomposition of ZIF-8 results in release of active components, synergistically enhancing the stroke therapeutic efficacy of CeO$_2$. (iv) CeO$_2$ @ZIF-8 also suppresses the inflammation- and immune response-induced injury by suppressing the activation of astrocytes and secretion of proinflammatory cytokines, thus achieving satisfactory prevention and treatment in neuroprotective therapy during ischemic stroke with high safety. As expected, this nanosystem effectively inhibits the lipid peroxidation in brain tissues of MCAO model mice, reducing the oxidative damage and apoptosis of neurons in brain tissue. Together, this study not only provides a new in situ synthetic approach of synergistic nanotherapeutics by using ZIF as bioactive surface decoration and CeO$_2$ NPs as functional core but also sheds light on the neuroprotective application mechanisms against reperfusion-induced injury in ischemic stroke.

**Synthesis and characterization of CeO$_2$ @ZIF-8**

First, to obtain CeO$_2$ nanopolyhedra, 0.05 M Ce(NO$_3$)$_2$·6H$_2$O and 0.01 M NaOH were dissolved in 40-mL deionized water and magnetically stirred until fully dissolved. Then, the mixture solution was transferred to a Teflon bottle and held in a stainless steel vessel autoclave. The autoclave was transferred into thermal treatment for 24 hours at 180°C. The products were washed by deionized water three times at 12,000 rpm, and the CeO$_2$ nanopolyhedra were obtained. Successively, 3.5 mg of CeO$_2$ nanopolyhedra and 100 mg of PVP were dissolved with 5-mL methanol solution, stirred at room temperature for 24 hours, collected by centrifugation, and stored in 100 μL of methanol to obtain PVP-CeO$_2$. Then, 3.5 mg of PVP-CeO$_2$ was added into 5 mL of 2-methylimidazole (25.6 mM, dissolved in methanol solution) and stirred for 15 min, and then 5 mL of 25.2 mM Zn(NO$_3$)$_2$·6H$_2$O methanol solution was added in this mixed solution and stirred for another 20 min at room temperature. The products were centrifuged at 12,000 rpm for 10 min and washed with methanol three times. Last, the CeO$_2$ @ZIF-8 nanomaterials were obtained by vacuum drying.

The as-synthesized CeO$_2$ nanopolyhedra, ZIF-8, and CeO$_2$ @ZIF-8 nanomaterials were characterized by different microscopic and spectroscopic analyses. Briefly, TEM (Hitachi H-7650, 100 kV), scanning electron microscope (Zeiss EVO 18, 20 kV), and high-resolution TEM (JEOL 2010) were used to characterize their morphology. Meanwhile, the size distribution and zeta potential were detected by Zetasizer Nano ZS. The Fourier transform infrared spectroscopy (Equinox 55, Bruker), UV–vis–NIR (near-infrared) spectrophotometry (UV-4150 Spectrophotometer, Hitachi), and Raman spectra (LabRAM HR Evolution, HÖRIBA) were used to determine the chemical composition of these nanomaterials. XRD pattern and XPS spectra were conducted using MSAL XD-2 x-ray diffractometer and Thermo ESCALAB 250Xi, respectively. Scavenging free radical ability was detected by EPR (A300, Bruker) and fluorescence spectrum (Lumina Fluorescence, Thermo Fisher Scientific).

**ABTS free radical scavenging assay**

ABTS scavenging assay was used to evaluate the antioxidant activity of CeO$_2$, ZIF-8, and CeO$_2$ @ZIF-8. Briefly, the ABTS free radical (ABTS$^+$) was formed by ABTS stock solution (5 mM, dissolved in PBS) reacted with manganese bioxide solution according to the previously described method (38). The different concentrations of CeO$_2$, ZIF-8, and CeO$_2$ @ZIF-8 were mixed with ABTS$^+$ radical solution, and then the absorbance at 734 nm within 60 min was examined using a cell imaging multi-mode reader (Cytation 5, BioTek Instruments Inc.).

**Scavenge H$_2$O$_2$ by in situ Raman spectra**

CeO$_2$, ZIF-8, and CeO$_2$ @ZIF-8 nanomaterials (10 mg) were dried in desiccator at room temperature for 12 hours and transferred to a clean glass sheet, where 50 μL of 10% H$_2$O$_2$ was added in the surface. Under the excitation of 488-nm laser, the samples were measured with a laser Raman microscope in the time range of 0, 20, and 40 min. H$_2$O$_2$ was then dropped into the same location of the sample immediately.

**Scavenge ‘OH and 'O$_2$ by EPR spectra analysis and fluorescence spectrum**

First, 'OH was generated through Fenton reaction with Fe$^{2+}$/H$_2$O$_2$ system by 1.8 mM FeSO$_4$ and 5 mM H$_2$O$_2$ for 10 min. In addition,
reverse damage of CeO$_2$@ZIF-8 to PC12 cells induced by t-BOOH.

The cellular uptake of CeO$_2$@ZIF-8 in PC12 cells was further investigated using DMPO-NMR. Briefly, PC12 cells (6 × 10$^5$ cells/ml) were added for another 48 hours. Then, the treated cells were further incubated with or without CeO$_2$@ZIF-8 at 4 μg/ml for another 24 hours. After rinsing three times with cold PBS, the cells were examined on mitochondrial morphology under a fluorescence microscope (EVOS FL, 100× objective lens).

Evaluation of intracellular ROS

Briefly, PC12 cells (2 × 10$^5$ cells/ml) were seeded in 96-well plates. After cell adherence, the PC12 cells were prestained with DCFH-DA for 30 min. Next, the cells were incubated with t-BOOH (15 μM) and different concentrations of CeO$_2$@ZIF-8 for 2 hours; meanwhile, the blank control group did not add drugs, and the other experiments were the same as the experimental group. The intracellular ROS generation was determined by a Cytation 5 reader (BioTek Instruments Inc.) at excitation and emission wavelengths of 488 and 528 nm, respectively.

Cell cycle and apoptosis analysis

The flow cytometric analysis was used to determine the cell cycle and apoptosis of PC12 cells induced by t-BOOH and recovered ability of CeO$_2$@ZIF-8. Briefly, PC12 cells were treated with 15 or 20 μM t-BOOH for 2 hours, and then the different concentrations of CeO$_2$@ZIF-8 were added for another 48 hours. Then, the treated cells were collected and fixed with 75% ethanol solution and stained with PI in the dark. The stained cells were analyzed on a Beckman CytoFLEX S flow cytometer.

For analysis of cell apoptosis, PC12 cells were plated in 100-mm dishes with a density of 4 × 10$^4$ cells/ml. After cell adherence, the cells were treated with t-BOOH (20 μM) and various concentrations of CeO$_2$@ZIF-8 for 48 hours at 37°C. Then, after removing the medium and washing with cold PBS, the cells were stained with annexin V and PI according to the experimental method in the assay kit (Solarbio) specification. Cell apoptosis was analyzed using a Beckman CytoFLEX S flow cytometer.

BBB assessment in vitro

In a previous study, HBMECs were used to establish the BBB model. Briefly, HBMECs were seeded in the upper chambers (3 μm, Corning, USA) with 1.5 × 10$^5$ cells and incubated for several days until the transendothelial electrical resistance reaches 330 ohm·cm$^2$. Then, the PC12 cells were then cultured in the lower chambers with a density of 8000 cells per well. After cell adherence, different concentrations of C6-CeO$_2$@ZIF-8 were added in the upper chambers for 120 minutes. The cell viability was determined by MTT assay (Cytation 5, BioTek Instruments Inc.) at excitation and emission wavelengths of 510 nm for ROS generation within 120 min using a cell imaging multimode reader at excitation and emission wavelengths of 488 and 528 nm, respectively.

Intracellular uptake of CeO$_2$@ZIF-8

For preparation of the C6-labeled CeO$_2$@ZIF-8, C6 was added in the PVP-CeO$_2$ reaction system before adding Zn(NO$_3$)$_2$·6H$_2$O with full stirring. The next step was similar to the preparation with CeO$_2$@ZIF-8. The cellular uptake of CeO$_2$@ZIF-8 in PC12 cells was measured on the basis of the fluorescence intensity of C6 loaded in CeO$_2$@ZIF-8. Briefly, PC12 cells (6 × 10$^5$ cells/ml, 2 ml) were seeded in 2-cm culture dishes and attached for 24 hours. The PC12 cells were further incubated with or without CeO$_2$@ZIF-8 at 4 μg/ml for another 24 hours. After rinsing three times with cold PBS, the cells were examined on mitochondrial morphology under a fluorescence microscope (EVOS FL, 100× objective lens).

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Internalization of CeO$_2$@ZIF-8 in PC12 cells by TEM

PC12 cells (1.5 × 10$^5$ cells/ml) were seeded in 10-cm dishes. After cell adherence, the cells were incubated with CeO$_2$@ZIF-8 (16 μg/ml)
for 6 hours at 37°C. After rinse with cold PBS and collection using trypsin digestion, the cells were fixed in 4% glutaraldehyde diluted in PBS over 24 hours and subsequently embedded in gelatin (2% gelatin in PBS). The cell mass was the size of a mung bean, wrapped with 1% agarose, and rinsed with 0.1 M PBS three times (each time 15 min). Then, 1% osmic acid–0.1 M PBS was fixed at room temperature (20°C) for 2 hours. The samples were dehydrated with 50, 70, 80, 90, 95, and 100% alcohol and 100% acetone in turn. Epoxy resin was used to bury samples, which were then kept in electric oven at 37°C overnight. Then, the epoxy resin was polymerized by changing the electric oven temperature to 60°C for 48 hours. The ultrathin sections (60 to 80 nm) were obtained with an ultrathin slicer, dyed in 2% uranyl acetate–saturated alcohol solution and lead citrate for 15 min, and then dried overnight at room temperature. Last, the sample was observed under TEM.

### In vivo protection against ischemic stroke

The MCAO mice model was established to evaluate the protection of CeO$_2$@ZIF-8 against ischemic stroke in vivo. Briefly, the MCAO model mice was formed by inserting the filament with silicone tip into the middle cerebral artery of the SD mice (250 to 300 g) for 90 min, and then the filament was removed to form the reperfusion. The sham-operated control group (10 mice) was established, consistent with the treatment of MCAO model except for the embolization. The MCAO model mice were randomly divided into three groups: control and CeO$_2$@ZIF-8 (0.2 mg/kg), and CeO$_2$@ZIF-8 (0.4 mg/kg) (10 mice per group). CeO$_2$@ZIF-8 was dispersed in saline solution and injected from the eyes of SD mice at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours, respectively. The serum samples were obtained by centrifugation and nitrification, and then the content of cerium was determined by ICP-MS. Last, data acquisition and the calculation of related pharmacokinetic parameters were realized by WinNonlin 3.3 software.

### Pharmacokinetic study of CeO$_2$ and CeO$_2$@ZIF-8

Eight female SD mice (250 to 300 g) were randomly divided into two groups and intravenously injected with CeO$_2$ and CeO$_2$@ZIF-8 at the dose of 0.4 mg/kg. Then, blood samples (~1 ml) were collected from the eyes of SD mice at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours, respectively. The serum samples were obtained by centrifugation and nitrification, and then the content of cerium was determined by ICP-MS. The main organs including heart, liver, spleen, lungs, kidney, and brain were removed, and the contents of cerium in these organs were examined by ICP-MS.

### Biodistribution of CeO$_2$@ZIF-8 in the main organs

Eight female SD mice (250 to 300 g) were randomly divided into two groups and intravenously injected with CeO$_2$ and CeO$_2$@ZIF-8 at 0.4 mg/kg (injection volume: 1 ml). After 72 hours, the main organs, including heart, liver, spleen, lungs, kidney, and brain, were removed, and the contents of cerium in these organs were examined by ICP-MS.

### Toxicity evaluation of CeO$_2$@ZIF-8 in vivo

The healthy C57 mice (~23 g) were used to evaluate the toxicity of CeO$_2$@ZIF-8 in vivo. The mice were randomly divided into three groups: control and CeO$_2$@ZIF-8 (0.4 and 0.8 mg/kg) (six mice per group). CeO$_2$@ZIF-8 was dispersed in saline solution and injected at the caudal vein (injection volume: 100 μl). After 2 weeks, serum samples were collected for hematological analysis, including blood glucose (GLU), cholesterol (CHOL), triglyceride (TG), high-density lipoprotein cholesterol (HDL-CH), low-density lipoprotein cholesterol (LDL-CH), alanine aminotransferase (ALT), aspartate transaminase (AST), total protein (TP), albumin (ALB), globulin (GLOB), albumin/ globulin (ALB/GLOB), uric acid (UA), creatinine (Cr), creatine kinase (CK), and lactate dehydrogenase (LDH). Meanwhile, the main organs (including heart, spleen, lung, liver, kidney, and brain) were collected for H&E staining and pathological analysis.

### Statistical analysis

Experiments were repeated at least in triplicate in this study, and all data were expressed as means ± SD. Multiple-group comparison and statistical analysis were performed using SPSS statistical program version 25 (IBM Corp., Armonk, NY) and one-way analysis of variance.
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**Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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Highly bioactive zeolitic imidazolate framework-8–capped nanotherapeutics for efficient reversal of reperfusion-induced injury in ischemic stroke
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