Supporting Information
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Ceria Nanoparticles that can Protect against Ischemic Stroke**
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Supporting Information

Experimental

Materials. Cerium acetate (98%), xylene (98.5%), hydrogen peroxide (H₂O₂, 30%), and rhodamine B isothiocyanate (RITC) were purchased from Sigma-Aldrich Inc (St. Louis, MO, USA). Oleylamine (approximate C18-content of 80–90%) was purchased from Acros Organics (Geel, Belgium). n-Hexane (99%), ethanol (99%), chloroform (99%), and acetone (99%) were purchased from Samchun Chemicals. 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (mPEG-2000 PE) and 1,2-distearyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG(2000) amine) were purchased from Avanti Polar Lipids Inc (AL, USA). SOD assay kit was purchased from Dojindo Laboratories (Japan) and amplex® red hydrogen peroxide/peroxidase assay kit was purchased from Molecular Probes Inc (Eugene, OR, USA).

Synthesis of ceria nanoparticles. One millimole (0.43 g) of cerium(III) acetate (98%, Sigma-Aldrich) and 12 mmol (3.25g) of oleylamine (approximate C18-content of 80–90%, Acros Organics) were added to 15 mL xylene (98.5%, Sigma-Aldrich). The resulting solution was stirred for 2 h at room temperature and then heated to 90°C under vacuum. One milliliter of deionized water was injected into the solution under vigorous stirring at 90°C, and the solution color changed to an off-white color, demonstrating that the reaction had occurred. The resulting mixture was aged at 90°C for 3 h to give a light yellow colloidal solution, which was then cooled to room temperature. Ethanol (100 mL) was added to the precipitated ceria nanoparticles. The precipitate was washed with ethanol and acetone using centrifugation, and the resulting ceria nanoparticles were easily dispersible in organic solvents, such as n-hexane and chloroform. For large-scale synthesis, we used a 10-fold increase in the volumes of these reagents.

Synthesis of phospholipid-PEG-capped ceria nanoparticles. To make biocompatible ceria nanoparticles, ceria nanoparticles dispersed in chloroform were encapsulated by a PEGL-phospholipid shell. First, 5 mL ceria nanoparticles in CHCl₃ (10 mg/mL) was mixed with 5 mL CHCl₃ solution containing 50 mg of mPEG-2000 PE. Then, solvents were evaporated by rotary evaporator and incubated at 80°C in vacuum for 1 h. The addition of 5 mL water resulted in a clear and light-yellowish suspension. After filtration, excess mPEG-2000 PE was removed using ultracentrifugation. Purified phospholipid-PEGL-capped ceria nanoparticles were dispersed in distilled water.

RITC-conjugated ceria nanoparticles. First, ceria nanoparticles dispersed in chloroform were encapsulated by amine-functionalized PEG lipid and PEGL-phospholipid shells. Five milliliters of ceria nanoparticles in CHCl₃ (10 mg/mL) was mixed with 5 mL CHCl₃ solution containing 45 mg mPEG-2000 PE and 5 mg DSPE-PEG(2000) amine. After evaporation of the solvent and subsequent filtration, ceria nanoparticles were dispersed in water. Amine groups were available for the attachment of RITC molecules. Five milligrams RITC was added, and the solution was stirred for 6 h. After ultracentrifugation, RITC-conjugated ceria nanoparticles were dispersed in distilled water.

Characterization of phospholipid-PEG-capped ceria nanoparticles. Transmission electron microscope (TEM) analysis was conducted using a JEOL JEM-2100 TEM operating at 200 kV. Samples were prepared by casting a drop of the ceria nanoparticle dispersion onto
a carbon-coated copper grid. XRD patterns were obtained using a Rigaku D/Max-3C diffractometer equipped with a rotation anode and a Cu Kα radiation source (λ = 0.15418 nm). Phase identification was performed using JCPDS-ICDD 2000 software (The International Centre for Diffraction Data; ICDD). XPS experiments were performed using a multipurpose surface analysis system (SIGMA PROBE, Thermo, UK). The photoelectron spectra were excited by an Al Ka (1486.6 eV) anode operating at 100 W. The base pressure during XPS analysis was maintained at less than 10⁻⁹ mbar, and the binding energy scale was calibrated from the C 1s peak at 285 eV. The 3d peak positions of ceria were then fitted using PeakFit (version 4.0) software (Systat Software, Chicago, IL, USA). UV/Vis absorbance measurements were taken using a JASCO V-550 instrument. DLS measurements were obtained using a particle size analyzer (ELS-Z, Otsuka Electronics, Japan). Elemental analysis was performed using inductively coupled plasma atomic emission spectrometer (ICP-AES, Shimadzu ICPS-1000IV-JAPAN) and an inductively coupled plasma mass spectrometer (ICP-MS, ELAN 6100, Perkin-Elmer SCIEX). The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was conducted using a Multiple Plate Reader (Victor3, manufactured by Perkin Elmer, USA).

**SOD mimetic activity assay.** The superoxide anion scavenging activity was assessed with SOD assay kit (Dojindo Laboratories, Japan). First, ceria nanoparticle solutions of different concentrations (0, 0.2, 0.4, 0.8, and 1.6mM) were mixed with 200 µL of WST-1 working solution. The reaction was initiated with the addition of 20 µL of xanthine oxidase solution. After incubating plate at 37°C for 20 min, the absorbance at 450 nm was measured using a micro plate reader (Victor3). Since the absorbance is proportional to the amount of superoxide anion, the SOD mimetic activity could be measured by quantifying the decrease of the color development at 450 nm.

**Catalase mimetic activity assay.** Quenching activities of hydrogen peroxide were quantified using Amplex® Red Hydrogen Peroxide/Peroxidase assay kit (Molecular Probes Inc.). Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) reacts with H₂O₂, in the presence of horseradish peroxide (HRP), to produce the red fluorescent resorufin. The florescence of resorufin (excitation at 571nm and emission at 585nm) indicates the H₂O₂ levels in the samples. First, a H₂O₂ standard curve was prepared and used to determine H₂O₂ concentration in each sample. After pipetting 50µL of ceria nanoparticle solutions of different concentrations (0, 0.125, 0.25, 0.75, and 1.5mM) into each micro well, 2µM of H₂O₂ solutions in the final concentration were added to nanoparticle samples and pre-incubated for 5 minutes. 50µL of the Amplex® Red reagent/HRP working solution was added and reactions were initiated. The fluorescence was measured after incubating for 30 min with protection from light.

**Autocatalytic activity of ceria nanoparticles.** The autocatalytic activity of ceria nanoparticles was examined by color change and UV/Vis spectroscopy. A drop of H₂O₂ solutions with various concentrations (0, 0.1, 0.5, and 1 M), as a source of hydroxyl radicals to mimic in vivo oxidative stress, were added to PBS solutions containing 5 mM ceria nanoparticles. As a control, nanoparticle dispersion without H₂O₂ was used. When more H₂O₂ was added to the ceria nanoparticle dispersion, the solution color changed from light yellow to orange, demonstrating that more Ce⁴⁺ species were generated by the addition of H₂O₂ (Figure S3a). In UV/Vis spectroscopy, upon the addition of H₂O₂, the shoulder between 300 and 400 nm was significantly red-shifted (Figure S3b). This color change and absorption shift was derived from the oxidation of Ce³⁺ species on the particle surface to Ce⁴⁺ by H₂O₂.
During the following 3 weeks, the H$_2$O$_2$ in the solution decomposed, and the observed orange color disappeared because the Ce$^{3+}$ regeneration occurred. The subsequent addition of H$_2$O$_2$ converted the light yellow color to orange again. This reversible autocatalytic activity of ceria nanoparticles is the key to their potential biomedical application as antioxidants in vivo.

**Cell culture.** Mammalian CHO-K1 cells were grown in monolayers in 89 % RPMI 1640 with L-glutamine (300 mg/L), 25 mM HEPES, and 25 mM NaHCO$_3$ supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and penicillin/streptomycin (100 U/mL and 100 µg/mL, respectively; Gibco) in a humidified 5% CO$_2$ atmosphere at 37 °C. Cells were cultured in 80-cm$^2$ flasks overnight to 80–90 % confluence.

**Fluorescence microscopy of cellular uptake.** To observe the cellular uptake of ceria nanoparticles, CHO-K1 cells were cultured in a 6-well plate (nonpyrogenic; BD Falcon, BD Bioscience, San Diego, CA, USA). Next, 200 µL ceria nanoparticles (0.1 mgCe/mL=0.7 mM) was added to each well, and the cells were incubated. After 1 h, the cells were washed with PBS 2 times, fixed with 2.5% paraformaldehyde, and stained with DAPI (2 µg/mL in PBS, Sigma-Aldrich). Red fluorescence was detected under the fluorescence microscope with an excitation of 350 nm and an emission of 470 nm for DAPI and with excitation of 540 nm and an emission of 625 nm for RITC.

**Intracellular ROS and cell viability assay.** CHO-K1 cells were initially seeded in triplicate in 96-well plates at a density of 25,000 cells per well and were grown in culture medium for 24 h at 37°C. Cells were washed with PBS 2 times, and the 1 mM tBHP solution was added, and incubated for 1 h. After washing with PBS, medium alone or medium containing ceria nanoparticles (0.125 or 0.25 mM) was added into each well. Six hours after treatment, an MTT assay (Sigma-Aldrich) was performed for viability. Twenty microliters of a 5 mg/mL solution of MTT was added to each well, and cells were incubated for additional 2 h at 37°C in a humidified 5% CO$_2$ atmosphere. The supernatant was aspirated, and the cells were dissolved in 200 µL DMSO. After shaking for 30 minutes, the absorbance was measured at 540 nm using a 96-well plate reader (Victor3).

**Fluorescence-activated cell sorting (FACS) analysis.** Cells were stained with FITC-conjugated ceria nanoparticles. Using BD FACS Calibur (BD Bioscience), we obtained relative optical densities of cells which absorbed fluorescent dye-conjugated ceria nanoparticles in various concentrations and time.

**Animals.** Male Sprague-Dawley rats (Koatech, Seoul, Republic of Korea), each weighing between 200 and 220g, were used in these experiments. All animal studies were carried out according to the National Institutes of Health Guide of the Institutional Animal Care and Use Committee of the Biomedical Research Institute at Seoul National University Hospital. Every effort was made to minimize animal suffering and to limit the number of animals used.

**Focal ischemia-reperfusion model and the injection of ceria nanoparticles.** Focal cerebral ischemia-perfusion was induced with a minor modification of the endovascular internal carotid artery (ICA) suture method developed by Longa et al.$^{[S1]}$ After inhalation anesthesia using 3% isoflurane in 30% oxygen and 70% air, the left common carotid artery (CCA) was exposed at its bifurcation using a midline cervical incision. The external carotid artery (ECA), ICA, and CCA were ligated using a 5-0 silk suture. The CCA was then transected, and a 5-0 nylon monofilament suture (with its tip rounded by heating) was inserted into the CCA. To occlude the origins of the MCA and proximal anterior cerebral artery, the suture was advanced into the ICA for a distance of 20 mm. The suture was secured in place using a ligature, and the wound was closed. The monofilament was removed 60 min after the occlusion. The animals were allowed food and water ad libitum. Seizure events were not
observed during the experiments at any time after the MCA occlusion. Rectal temperature was maintained at \(37\pm0.5^\circ C\) using a thermistor-controlled heating blanket (Figure S9). We administered various doses of ceria nanoparticles (0.1, 0.3, 0.5, 0.7, 1.0, and 1.5 mg/kg) or PBS intravenously immediately after reperfusion to detect optimal doses.

**Measurement of infarct volumes.** After cardiac perfusion-fixation with 4% paraformaldehyde in 0.1 mol/L PBS, brains were removed quickly and cut into 30-µm-thick coronal sections on a freezing microtome. Ten brain sections were mounted onto glass slides, and processed for Nissl staining. Infarct volumes were measured using an image analysis program, ImageJ (National Institutes of Health, Bethesda, MD).

**In situ labeling of DNA fragmentation.** Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) was performed with the use of a commercially available kit as described previously.\[^{[S2]}\] Sections were incubated in a TdT-labeling reaction mixture for 90 min, colored with DAB solution, and counterstained with methyl green. A single axial section through the center of the hemorrhagic lesion was analyzed. Eight sampling regions were placed along the periphery. TUNEL-positive cells were identified and counted. Total counts in these sampling regions were converted into cell densities for quantification and comparison between the treatment groups. According to morphological criteria,\[^{[S3]}\] apoptosis-specific TUNEL-positive nuclei with chromatin condensation and fragmented nuclei (>2 apoptotic bodies) were considered probable apoptotic cells, while nonapoptotic TUNEL-positive cells exhibited diffuse light labeling of nuclei (Figure S14).

**Biodistribution of ceria nanoparticles.** At 24 h after injection of ceria nanoparticles, rats with focal cerebral ischemia were decapitated to rapidly harvest the brain and other organs. Post-mortem samples were obtained to determine ceria concentrations in the brain, heart, kidneys, liver, and blood, using inductively coupled plasma-mass spectrometry (ICP-MS).

**ICP-MS analysis.** Determination of cerium content in organs was performed by ICP-MS analysis (ELAN 6100, Perkin-Elmer SCIEX). Organs were dissolved in aqua regia. The resulting solutions were diluted in HNO\(_3\) (2%, 2 ppb, 1:300 v/v for the brain, heart, spleen, lung, liver, and kidneys).

**Tracking of ceria nanoparticles.** After 60 min of occlusion-reperfusion, RITC-conjugated ceria nanoparticles were injected intravenously. At 6 h after ceria injection, rats were sacrificed and the brains were examined under a confocal laser scanning biological microscope (LSM 410 META; Carl Zeiss, Jena, Germany). To evaluate the distribution of ceria nanoparticles in the ischemic brain, we conducted computerized visual augmentation using 3-dimensional reconstruction of the fluorescence signals. After adding 1,250 high-power field images, a raw image of the whole brain was created (Figure S16a). Theses raw images were imported from the LSM Image Examiner Version 4.0 (Carl Zeiss MicroImaging GmbH, Germany). Visible sediments on scanned images were manually excluded using Photoshop (Adobe Systems Inc., USA), and images were then converted by the ImageJ 1.44 (NIH, Bethesda, Maryland, USA) plugin Auto Threshold, which converts 8-bit images to binary images, to facilitate automatic particle filtering. To suppress the remaining background noise, we used the “Analyze Particles” mode in ImageJ (Figure S16b). Extracted (x,y) data values from the processed images were computed to save kernel density estimates in SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) (Figure S16c). Calculated (x,y,density) data values were then reincorporated to generate 3-dimensional contour plots in
ImageJ (Figure S16d). We then overlapped original rat brain images with scatter plots of ceria nanoparticles drawn by R version 2.14 (Figure S16e).

**Detection of ROS in vivo.** Production of ROS during cerebral ischemia was investigated using a previously reported hydroethidine (HEt) method. An HEt solution (100 µL, 20 mg/mL in DMSO; Sigma-Aldrich) was administered 15 min before the induction of ischemia. Animals were killed 4 h after the induction of ischemia using transcardial perfusion. Fluorescence was assessed microscopically at an excitation wavelength of 355 nm and an emission wavelength of greater than 415 nm for HEt detection or at an excitation wavelength of greater than 510–550 nm and an emission wavelength of greater than 580 nm for oxidized HEt detection.

**Lipid peroxides assay.** Malondialdehyde was estimated as an indicator of lipid peroxidation. The malondialdehyde level in the brain tissue at 24 hrs after stroke was measured using the thiobarbituric acid reduction method with a commercially available kit (Cell Biolabs, San Diego, CA, USA).

**Western blot analysis.** Twenty-four hours after the induction of focal ischemia, rats were sacrificed by decapitation, and the brains were extracted. After centrifugation of hemisphere homogenates, 50 µg of protein was separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred onto a nitrocellulose membrane. Membranes were incubated in blocking buffer (5% skim milk in 50 mM Tris, pH 7.5, 0.15 mM NaCl, 0.05% Tween-20), and the blots were probed with antibodies for p53, phospho-p53, caspase-3, cleaved caspase-3, and gelsolin (Cell Signaling Technology, Danvers, MA). Immunoreactivity was visualized using enhanced chemiluminescence, and relative densities were determined by comparing measured values with mean values of the control group.

**Statistical analysis.** Values are presented as the mean ± standard deviation. Data were analyzed using the Student’s t-test for normally distributed unpaired samples. The nonparametric Mann-Whitney U test or Wilcoxon signed-rank test was used for unpaired or paired samples, respectively. A 2-tailed p-value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).
Figure S1. Hydrodynamic size distribution of ceria nanoparticles in PBS, as measured by dynamic light scattering (DLS). (a) DLS of water-dispersible ceria nanoparticles (mean diameter = 17.9 nm). (b) Photo of water-dispersible ceria nanoparticles. The clear solution indicated the lack of aggregation and the good dispersibility of the solution.

Figure S2. ROS-scavenging activities of ceria nanoparticles by superoxide dismutase-mimetic assay and catalase-mimetic assay. (a) Dose-dependent $\text{O}_2^-$ scavenging by ceria nanoparticles. (b) Quenching activities of hydrogen peroxide by ceria nanoparticles. Ceria nanoparticles exhibit catalase mimetic activity at physiologically relevant hydrogen peroxide concentrations.
Figure S3. Autocatalytic activity of ceria nanoparticles as examined by color change and UV/Vis spectroscopy. (a) Reversible color change, demonstrating that Ce$^{4+}$ species were generated by the addition of a hydrogen peroxide (H$_2$O$_2$) solution to the ceria nanoparticles. (b) UV/Vis absorption spectra show the red-shift of the band between 300 and 400 nm after the addition of H$_2$O$_2$ solution to 5 mM ceria nanoparticle suspensions.

Figure S4. Protective effects of ceria nanoparticles against ROS-induced cell death in vitro. To investigate the effects of ceria nanoparticles on ROS-induced cell death in vitro, CHO-K1 cells were incubated with tert-butyl hydroperoxide (tBHP), which increases intracellular ROS (n = 3 each). In an MTT assay, the addition of 1 mM tBHP significantly increased cell death compared to the control (83%, p < 0.05), while the addition of 0.125 mM ceria nanoparticles increased cell viability (113%, p < 0.05).

Figure S5. Fluorescence microscopy images of CHO-K1 cells incubated with ceria nanoparticles conjugated with a fluorescent rhodamine dye. (a) The nuclei were stained blue with 4',6-diamidino-2-phenylindole (DAPI). (b) Red fluorescence from the nanoparticles was detected throughout the cytoplasm. (c) Overlapped images from (a) and (b) represent the cellular uptake and intracellular localization of ceria nanoparticles.
Figure S6. Cellular uptake of ceria nanoparticles according to concentration and time. (a) The cells were cultured in a 6-well plate and various concentrations of FITC-conjugated ceria nanoparticles were added to each well and incubated. After 60 min, the cells were washed with PBS 2 times, and intensity of the green fluorescence on the cell was detected using fluorescence-activated cell sorting (FACS). For 3 nm (core size) ceria nanoparticles, the uptake in cells was increased with dose dependent manner until 0.125 mM, and above 0.125 mM, the uptake of ceria nanoparticles was saturated (* compared with control, # compared with 0.025 mM, \( p < 0.05 \)). (b) The uptake of 3 nm (core size) ceria nanoparticles was increased with time-dependent manner until 180 min (*, \( p < 0.05 \) compared with control).

Figure S7. The MTT assay for cell death after exposing ceria nanoparticles. Above 0.25 mM, survival rates of normal cells were decreased, it is well correlated with previous studies (*, \( p < 0.05 \)).\(^ {55, 6} \)
Figure S8. Model of focal ischemia. (a, b) After inhalation anesthesia using 3\% isoflurane in 30\% oxygen and 70\% air, the left common carotid artery (CCA) was exposed at its bifurcation by a midline cervical incision. (c) The external carotid artery (ECA), internal carotid artery (ICA), and CCA were ligated with a 5-0 silk suture. (d) The CCA was then transected, and a 5-0 nylon monofilament suture (with its tip rounded by heating) was inserted into the middle cerebral artery (MCA). (e, f) To occlude the origins of the MCA and proximal anterior cerebral artery, the suture was advanced into the internal carotid artery for a distance of 20 mm beyond the ICA. (g) The suture was secured in place with a ligature, and the wound was closed. The monofilament was removed 60 min after the occlusion. (h) After recovery, the animals were allowed food and water ad libitum.
Figure S9. Protocols for our in vivo studies. (a) Routine protocols were performed to check infarct volume, TUNEL-positive cells, western blots (WBs), and tissue concentrations. (b) For tracking of ceria nanoparticles, RITC-conjugated ceria nanoparticles were injected intravenously. (c) To investigate the in vivo antioxidant effects of ceria nanoparticles, we used hydroethidine (HEt) solution, which is converted into a red-colored form after being oxidized by reactive oxygen species.
**Figure S10.** Adjusted infarct volume according to individual brain size. To adjust infarct volume according to individual brain size, we calculated the percentage of infarct volume relative to the volume of the contralateral hemisphere (n = 12 each, except for 0.1 and 1.5 mg/kg doses, where n = 6). In animals treated with low doses of ceria nanoparticles (0.1 and 0.3 mg/kg), the ratios of infarct volume to total volume were not different between the groups, while in animals treated with optimal doses of ceria nanoparticles (0.5 and 0.7 mg/kg), the ratios of infarct volume to total volume were significantly decreased (p < 0.05). However, in animals treated with higher doses (1.0 and 1.5 mg/kg), a decrease in this ratio was not observed.

**Figure S11.** Regional analysis of infarct volume. To investigate the region in where the infarct volume was decreased the most, we investigated infarct volume in 2 separated regions (the cortex and infarct core). (a) In the cortex of the brain, the infarct volume decreased significantly when optimal doses of ceria nanoparticles (0.5 and 0.7 mg/kg) were injected. (b) However, in the infarct core, infarct volumes did not decrease after treatment the various doses of ceria nanoparticles. Based on these results, we postulated that the main site of action for ceria nanoparticles was the brain cortex, which is consistent with our results demonstrating the location of ceria nanoparticles within the animal.
Figure S12. Biodistribution of relatively higher doses of ceria nanoparticles. (a, b, c, d) After stroke, neuronal cells absorbed RITC-conjugated ceria nanoparticles compared with the ceria-injected group without stroke. The number of ceria-stained cells and the intensity of fluorescence in each cell were increased with dose dependent manner in 0.5, 1.0, 1.5 mg/kg of ceria nanoparticles. Scale bar = 50 µm. (e) After quantitative analysis using ICP-MS, the concentration of ceria nanoparticles in infarcted hemisphere of brain was increased with dose dependent manner, and the concentration significantly increased at the higher dose (1.5 mg/kg) compared to lower dose of 0.5 mg/kg (*, p < 0.05).
Figure S13. Morphological criteria of apoptotic TUNEL-positive cells. According to morphological criteria, apoptosis-specific TUNEL-positive nuclei with chromatin condensation and fragmented nuclei (≥ 2 apoptotic bodies) were considered apoptotic cells (arrow), and non-apoptotic TUNEL-positive cells exhibited diffuse light labeling of nuclei. TUNEL-negative cells were counter-stained with methyl green (blue). In the cortical area, the number of apoptotic TUNEL-positive cells in the ceria-injected group was lower than in the control (Figure 4 in the main text). Scale bar = 50 µm.
Figure S14. Computerized visual augmentation with 3-dimensional reconstruction. (a) At 6 h after injection of RITC-conjugated ceria nanoparticles, the rats were sacrificed, and the brains were examined under a confocal laser scanning biological microscope. After adding 1,250 high-power field images, a raw image of whole brain was created. (b) Visible sediments on scanned images were manually excluded, and the images were then converted using the ImageJ plugin Auto Threshold, which converts 8-bit images to binary images, to facilitate automatic particle filtering. (c) Extracted (x,y) data values from the processed images were computed to save kernel density estimates in SAS software. (d) Calculated (x,y,density) data values were then reincorporated to generate 3-dimensional contour plots in ImageJ. (e) Original rat brain images were overlapped with scatter plots of ceria nanoparticles drawn by R version 2.14.
Table S1. Surface reactivity of ceria nanoparticles.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>BET surface area [m²/g]</th>
<th>Amount of surface of nanoparticles [10^3 m²/mL]</th>
<th>Quenched peroxide levels [µM]</th>
<th>Surface reactivity of ceria nanoparticles for 5 min [µmol/m²]</th>
<th>Surface reactivity of ceria nanoparticles per min [nmol/m²•min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125 mM ceria nanoparticles</td>
<td>141</td>
<td>3.1</td>
<td>0.53</td>
<td>0.17</td>
<td>34</td>
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<tr>
<td>0.25 mM ceria nanoparticles</td>
<td>141</td>
<td>6.2</td>
<td>0.73</td>
<td>0.12</td>
<td>24</td>
</tr>
</tbody>
</table>

Because surface reactions mostly occur on the core surface, we first measured the specific surface area of the ceria nanoparticle core. The surface area of ceria nanoparticles after removing the stabilizing organic surfactants, measured using Brunauer-Emmet-Teller (BET) method, was 141 m²/g. From the catalase assay in figure S2b in the supporting information for 5 min, we can estimate quenched hydrogen peroxide levels per time and per specific surface areas of ceria nanoparticles during exposure to biological tissues from the following table. It is expected to be about 30 nmol/m²•min. From ICP-MS data in Figure 3a in the main text, we can get the concentration of ceria nanoparticles after injection of 0.5 mg/kg ceria nanoparticles. The average concentration of ceria nanoparticles was 0.8 µg/g, and average hemispheric brain weight (half brain) of rats is ~ 1 g. Consequently, the total weight of ceria nanoparticles in infarcted hemisphere was estimated to be 0.8 µg. From the BET model, the amount of surface area in hemispheric stroke brain was 0.8 µg × 141 m²/g = 1.13 × 10⁴ m². The estimated quenching activity of cerium oxide is 1.13 × 10⁴ m² × 30 × 10⁹ mol/m²•min × 60 min/hrs × 24 hrs = 4.9 nmol. In our experiment using lipid peroxide assay, the concentration of ROS was decreased from 24.6 µM to 15.5 µM. The difference between two values was 9.1 µM and the volume of stroke area was about 0.15 mL (Figure 2a in main text). Therefore, the amount of removed ROS by 0.5 mg/kg ceria nanoparticles was 9.1 µM × 1.5 × 10⁴ L = 1.4 nmol. The amount of real ROS-quenching activity of ceria nanoparticles (1.4 nmol) was lower than that of estimated quenching effect (4.9 nmol) in in vivo model. Because brain is not liquid solution, the ROS-producing area is not always correlated with ceria-distributed area, and the maximal effect of ceria nanoparticles was not shown. From this reason, the calculated quenching activity of 0.5 mg/kg ceria nanoparticles may be overestimated.
References for the Supporting Information