Prophylactic Edaravone Prevents Transient Hypoxic-Ischemic Brain Injury
Implications for Perioperative Neuroprotection

Yu-Yo Sun, PhD*; Yikun Li, PhD*; Bushra Wali, PhD; Yuancheng Li, PhD; Jolly Lee, BS; Andrew Heinmiller, MS; Koji Abe, MD, PhD; Donald G. Stein, PhD; Hui Mao, PhD; Iqbal Sayeed, PhD; Chia-Yi Kuan, MD, PhD

Background and Purpose—Hypoperfusion-induced thrombosis is an important mechanism for postsurgery stroke and cognitive decline, but there are no perioperative neuroprotectants to date. This study investigated whether prophylactic application of Edaravone, a free radical scavenger already used in treating ischemic stroke in Japan, can prevent infarct and cognitive deficits in a murine model of transient cerebral hypoxia-ischemia.

Methods—Adult male C57BL/6 mice were subjected to transient hypoxic-ischemic (tHI) insult that consists of 30-minute occlusion of the unilateral common carotid artery and exposure to 7.5% oxygen. Edaravone or saline was prophylactically applied to compare their effects on cortical oxygen saturation, blood flow, coagulation, oxidative stress, metabolites, and learning-memory using methods that include photoacoustic imaging, laser speckle contrast imaging, solid-state NMR, and Morris water maze. The effects on infarct size by Edaravone application at different time points after tHI were also compared.

Results—Prophylactic administration of Edaravone (4.5 mg/kg×2, IP, 1 hour before and 1 hour after tHI) improved vascular reperfusion, oxygen saturation, and the maintenance of brain metabolites, reducing oxidative stress, thrombosis, white-matter injury, and learning impairment after tHI insult. Delayed Edaravone treatment after 3 h post-tHI became unable to reduce infarct size.

Conclusions—Acute application of Edaravone may be a useful strategy to prevent postsurgery stroke and cognitive impairment, especially in patients with severe carotid stenosis. (Stroke. 2015;46:1947-1955. DOI: 10.1161/STROKEAHA.115.009162.)

Key Words: antioxidants ■ MCI-186 ■ stroke ■ thrombosis

Stroke, a dangerous complication of surgery, occurs in 5% to 8% of carotid endarterectomy and cardiac procedures and is more common in patients with symptomatic carotid stenosis.1–4 A high incidence of silent brain infarct (≈25%) and cognitive decline (33% to 83% in short term and 42% by 5 years) also occurs after coronary artery bypass grafting.5,6 Studies suggest that transient ischemia triggers a sustained prothrombotic tendency in the cerebral vasculature.7 The addition of hypoxia to ischemia further accelerates coagulation and leads to infarction.8–11 Yet, because of the risk of bleeding, anticoagulants are unsuitable for perioperative neuroprotection. To date, there is no specific drug to prevent postsurgery stroke and cognitive deficits.12

Edaravone is a free radical scavenger that readily crosses blood–brain barrier.13,14 It has been used for treating acute ischemic stroke in Japan since 2001 and is in clinical trials in Europe.15,16 When combined with tissue-type plasminogen activator, Edaravone confers synergistic benefits in both animal models (reduction of the mortality and infarct size) and stroke patients (a greater recanalization rate).9,17 Research indicates that Edaravone markedly reduces oxidative stress in multiple components of the neurovascular unit, including neurons, platelets, endothelial cells, and pericytes.18–21 to interrupt the Virchow’s triad of thrombosis (the stasis of blood flow, endothelial injury, and hyper-coagulability).11 Hence, prophylactic administration of Edaravone...
may confer perioperative neuroprotection, but this utility is yet to be tested.

To this end, we examined the effects of prophylactic Edaravone treatment in a transient hypoxia-ischemia (tHI)-induced thrombotic stroke model. In this model, the unilateral common carotid artery of adult mice is occluded when the animal inhales hypoxic gas (7.5% oxygen) via a face-mask. We have shown that as brief as 30 minutes tHI induces thrombosis, reperfusion deficits, and cortical infarct. In the present study, we examined the effects of 2 doses of Edaravone (1 hour before and 1 hour after tHI) to mimic pre- and postoperative care for patients and evaluated the therapeutic window of this treatment.

Our results showed that pre- and acute post-tHI application of Edaravone mitigates brain injury and learning-memory deficits, suggesting that Edaravone is a promising neuroprotectant against poststroke surgery and cognitive decline. Further translational study of this prophylactic treatment merits consideration.

**Material and Methods**

**Stroke Surgery**

Male C57BL/6 mice and Thy1-YFP mice at the age of 10 to 13 weeks were subjected to transient cerebral HI, as previously described, with minor modifications. Animals weighing 22 to 30 g were anesthetized under 2% isofluorane to perform right common carotid artery occlusion (RCCAO) with 2 releasable knots of 4.0 silk sutures that were released after 1 hour of the hypoxic stress. After RCCAO, mice were infused with 7.5% O2/2.5% N2 via face-mask for 30 minutes, whereas the animal core body temperature was maintained at 37.5±0.5°C by a rectal thermoprobe coupled to a heating lamp. After tHI, mice were randomized for treatments, and investigators analyzing brain infarction were blinded to the treatments. Treatment consisted of intraperitoneal injection of 3 or 4.5 mg/kg Edaravone (a gift of the Mitsubishi Tanabe Pharma Cooperation, Osaka, Japan) at designated time-points in each regimen, which was illustrated in the corresponding figure in the result section. The number of operated animals, the mortality, the outliers (>2 SD), and animals for quantification of infarction were also tabulated. All procedures were approved by the Institutional Animal Care and Use Committee and conform to the National Institutes of Health Guide for Care and Use of Laboratory Animals, as well as the Animals in Research: Reporting In-Vitro Experiments guidelines.

**Post-Surgery Monitoring and Measurement of Brain Infarction**

Detection of infarction was performed by in vivo TTC staining at 24 h after tHI by a laboratory member unaware of the treatment. Brains were sectioned into 0.7 mm thickness slices (8 per brain) and analyzed using the ImageJ 1.4 software (NIH, Bethesda, MD). The infarct size was quantified as the ratio of the infarcted area to the total area of the uninjured, contralateral hemisphere.

**Laser Speckle Contrast Imaging and Cortical Oxygen Saturation Measurement**

Cortical oxygen saturation (SaO2) was measured using the tissue oxygenation monitoring system (mooRVSMS-OXY™; Moor Instruments Inc. DE). Briefly, anesthetized mice with skull exposed were measured in both cerebral hemispheres (2 mm posterior and 2 mm lateral to Bregma) using optic probe and recorded immediately after the RCCAO surgery, RCCAO plus hypoxia (30 minutes), and 1 h post-tHI. Cerebral blood flow (CBF) measurement was performed by a 2-dimensional laser speckle contrast imaging system following the manufacturer’s instruction (MoorFLPI-2; Moor Instruments Inc.). Briefly, poststroke mice at 2 h post-tHI were reanesthetized and placed in the prone position with the skull exposed yet unopened. CBF were measured in both hemispheres and recorded continuously for at least 7 minutes. The CBF images are shown with arbitrary units in a 16-color palette by the MoorFLPI software. SaO2 and CBF were quantified as the percentage to the contralateral hemisphere.

**Immunohistochemistry and Immunoblot**

Immunohistochemistry and immunoblotting were performed as previously described. The following antibodies were used: rabbit anti-fibrinogen (a gift of Dr J. Degen), Alexa Fluor 488 isolectin GS-IB4 conjugate (#I21411, Invitrogen, Carlsbad, CA), rabbit anti-ERG (No 92513, Abcam, Cambridge, MA), and mouse anti-p-actin (Sigma, St Louis, MO).

**Histological Assay of Vascular Perfusion and White-Matter Injury**

Evans blue albumin was used to evaluate vascular perfusion and blood–brain barrier leakage as previously described. Vascular perfusion was quantified using the CellSens software (Olympus, Tokyo, Japan) with adjusted threshold and background to obtain the total intensity of vasculature. The value of ipsilateral vasculature intensity was normalized to the contralateral hemisphere. To detect white-matter injury, Thy1-YFP-labeled nerve fiber and the FluoroMyelin Red (Molecular Probes, Invitrogen) in the external capsule were measured using CellSens (Olympus).

**Detection of Oxidative Stress**

Lipid peroxidation was measured by quantifying malondialdehyde in brain extracts using a commercial kit (OxiSelect™; Cell Biolabs Inc., San Diego, CA), as previously described. The superoxide production was determined by oxidized hydroethidine detection (oxHET; Invitrogen), as previously described. Briefly, the HET solution (1 mg/mL) was injected through the tail vein to the animals at 30 minutes before killing. oxHET was detected at an emission of 590 nm and quantified as the ratio to DAPI-positive nuclei in 4 randomly selected visual fields (100x).

**High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance Study**

For ex vivo NMR analysis, tissue samples were prepared as previously reported. Each sample was cut from the snap-frozen tissue slice and thawed in 99.996% saline deuterium oxide (Sigma) before loading to the sample holder/rotor (4 mm ZrO2). 99.996% deuterium oxide containing 0.75% 3-(trimethylsilyl) propionic acid was added to obtain a frequency-lock signal for NMR and to serve as an internal reference for chemical shift and concentration measurements.

Solid-state high-resolution magic angle spinning (HRMAS) NMR experiments were conducted, as previously reported, using a Bruker AVANCE 600 WB NMR spectrometer (Bruker Instruments, Inc., Billerica, MA) with a dedicated 4 mm HRMAS probe. Sample spinning rates were controlled in the range of 2800 KHz (±2 Hz) or at the lower spin rate of 800 Hz if the rotor synchronized delay alternating with nutation for tailored excitation sequence. A rotor-synchronized Carr–Purcell–Meiboom–Gill pulse sequence was used to suppress broad signals from macromolecules. The 1-dimensional
NMR spectra were recorded using the repetition time of 5.0 s, the spectral width was 10 kHz, and the number of transients of 256. The presence and the level of selected brain metabolites were determined based on their chemical shifts in the spectra. Prophylactic Edaravone Prevents Reperfusion and Reoxygenation Deficits After tHI Insult

To mimic cerebral hypoperfusion and hypoxia possibly encountered in surgical procedures, we subjected adult C57BL/6 mice to a tHI insult that consists of unilateral occlusion of the common carotid artery and exposure to 7.5% oxygen for 30 minutes (Figure 1A). Color flow Doppler imaging was used to monitor blood flow changes in the different cerebral vessels.}

**Figure 1.** Transient hypoxia-ischemia (tHI) caused prolonged deficits in cortical oxygenation. **A.** A schematic diagram of the tHI model. **B.** Doppler flow imaging and 3D reconstruction showed reduced perfusion in the ipsilateral hemisphere and the reversal of blood flow in the right ICA (RICA) in the Circle of Willis after RCCA occlusion (RCCA0). **C.** Co-registered anatomic image (B-mode micro-ultrasound) and oxygen saturation (SaO2 in a red-white-blue color-map by photoacoustic imaging) showed bilateral difference of SaO2 between the left (L, contralateral) and right (R, ipsilateral) cerebral cortex of an adult C57BL/6 mouse after RCCAO in normoxic (20.9% O2) or hypoxic (7.5% O2) condition. **D.** After 30 minutes tHI, the ipsilateral cortex showed poorer recovery of SaO2 in either normoxia or 100% O2 condition. Shown is the representative response (n=3). ECA indicates external carotid artery; ICA, internal carotid artery; MCA, middle carotid artery; and RCCA, the right common carotid artery.
and 3D reconstruction showed that the RCCAO reduced blood flow in the ipsilateral hemisphere and reversed blood flow in the right internal carotid artery in the circle of Willis (Figure 1B). Photoacoustic imaging showed that oxygen saturation (SaO₂) in the ipsilateral cortex was reduced from \( \approx 70\% \) to \( \approx 61\% \) on RCCAO and plummeted to \( \approx 20\% \) on exposure to hypoxic air (Figure 1C). After 30 minutes tHI, the ipsilateral hemisphere showed poorer recovery of SaO₂ than the contralateral cortex (\( \approx 28\% \) compared with \( \approx 39\% \) in normoxia and \( \approx 53\% \) versus \( \approx 70\% \) under 100% oxygen; Figure 1D; \( n \geq 3 \) for each). This pattern suggests that a transient episode of HI may impair subsequent cortical oxygenation.²

Next we tested the effects of prophylactic Edaravone treatment (4.5 mg/kg×2, 1 hour before and 1 hour after tHI). Despite
comparable reduction of cortical $\text{Sa}_2$ under RCCAO and combined RCCAO-hypoxia insult, Edaravone-treated animals exhibited better recovery of cortical $\text{Sa}_2$, at 1 hour post-tHI ($P=0.012$, $n=8$–9; Figure 2A). Similarly, laser speckle contrast imaging showed greater recovery of CBF in Edaravone-treated than saline-treated animals at 2 h post-tHI (85% versus 64% of the contralateral hemisphere, $P=0.011$, $n=8$–9; Figure 2B). Tail-vein injection of Evans blue dye at 2 h post-tHI also showed better vascular perfusion in the ipsilateral cortex of Edaravone-treated than saline-treated mice (83% versus 43%, $P=0.006$, $n=4$; Figure 2C). Immunostaining showed greater fibrin(ogen) precipitation and immuno-reactivity to P-Selectin (a marker for endothelial activation) and GPIIb/CD41 (a platelet surface receptor) in the ipsilateral cortex (n=4, Figure 2C and data not shown). Immunoblot analysis also uncovered greater deposition of fibrin(ogen) in the ipsilateral cortex in saline-treated mice at 2 h after tHI (n=3, $P=0.04$ compared with untouched animals; Figure 2D). These data suggest that tHI induces thrombosis and reperfusion-reoxygenation deficit, which is attenuated by Edaravone treatment.

**Edaravone Abates tHI-Induced Oxidative Stress in the Brain Parenchyma and Vascular Wall**

Tissue ischemia accumulates succinate in the mitochondria, which drives superoxide formation on reperfusion by reverse electron transport.25 To detect superoxide, tHI-injured mice were intravenously injected with hydroethidine, which emit 590 nm fluorescence when it is oxidized (oxHET) by superoxide. This analysis showed far more oxHET-positive cells in the ipsilateral hemisphere in saline-treated mice than Edaravone-treated mice at 2.5 h after tHI (26.3% versus 5.3% of all DAPI+ nuclei, $P=0.002$, n=8; Figure 3A). Double-labeling with ERG (a marker for endothelial cell) showed colocalization with oxHET+ nuclei in the ipsilateral cortex of saline-treated, but not Edaravone-treated animals (arrows in Figure 3A and 3B). Saline-treated mice also exhibited greater increase of malondialdehyde, a marker of lipid peroxidation, than Edaravone-treated mice at 24 h after tHI (1.69 versus 1.30-fold, $P=0.0004$, n=5; Figure 3C). These results suggest that prophylactic Edaravone treatment ameliorates tHI-induced oxidative stress in both brain parenchyma and the vascular endothelium.

**Edaravone Prevents tHI-Induced Alteration of Brain Metabolites and Learning Deficits**

Depletion of N-acetyl-aspartate is a hallmark of brain damage after cerebral ischemia.26 Using HRMAS NMR analysis of tHI-injured tissue samples, we found that 30 minutes tHI produced progressive reduction of N-acetyl-aspartate in saline-treated mice from 6 to 24 h recovery (n=4 each, $P=0.034$ compared with untouched mice). In contrast, the level of N-acetyl-aspartate was suppressed at 6 h, but recovered at 24 h in Edaravone-treated mice (n=3, $P=0.032$ compared with saline-treated mice; Figure 4A). We also subjected Thy1-YFP mice to tHI insult and found attenuated white matter injury in the ipsilateral hemisphere after prophylactic Edaravone treatment ($P=0.024$, n=4; Figure 4B). Using FluoroMyelin dye, we also found reduction and diffusion of myelin in the external capsule (EC) of ipsilateral hemisphere in saline-treated mice at 24 h post-tHI (n=4; Figure 4B).

Next we compared the learning capacity in MWM between the sham and tHI-injured mice that received prophylactic saline or Edaravone treatment after 10 d recovery (n=12 each). In 5 consecutive days, sham and Edaravone-treated mice performed similar. In contrast, saline-treated mice showed significant impairment in learning compared to sham and Edaravone-treated groups (Figure 4C). These results suggest that Edaravone treatment prevents tHI-induced alterations in brain metabolism and learning deficits.

**Figure 3.** Edaravone abated oxidative stress in the brain parenchyma and vascular endothelium. **A**, Merged image of oxidized hydroethidine (oxHET) and isolectin B4 (an endothelial marker) labeling revealed more superoxide-positive cells in the ipsilateral hemisphere in saline-treated than Edaravone-treated mice at 2.5 h post-tHI (26.3% versus 5.3% of all DAPI+ nuclei, $P=0.002$, n=8; Figure 3A). Double-labeling with ERG (a marker for endothelial cell) showed colocalization with oxHET+ nuclei in the ipsilateral cortex of saline-treated, but not Edaravone-treated animals (arrows in Figure 3A and 3B). Saline-treated mice also exhibited greater increase of malondialdehyde, a marker of lipid peroxidation, than Edaravone-treated mice at 24 h after tHI (1.69 versus 1.30-fold, $P=0.0004$, n=5; Figure 3C). These results suggest that prophylactic Edaravone treatment ameliorates tHI-induced oxidative stress in both brain parenchyma and the vascular endothelium.

**Figure 4.** Edaravone prevented tHI-induced alterations in brain metabolites and learning deficits. **A**, Progressive depletion of N-acetyl-aspartate in saline-treated mice from 6 to 24 h recovery (n=4 each, $P=0.034$ compared with untouched mice). In contrast, the level of N-acetyl-aspartate was suppressed at 6 h, but recovered at 24 h in Edaravone-treated mice (n=3, $P=0.032$ compared with saline-treated mice; Figure 4A). **B**, Thy1-YFP mice showed attenuated white matter injury in the ipsilateral hemisphere after prophylactic Edaravone treatment ($P=0.024$, n=4; Figure 4B). **C**, MWM learning deficits were attenuated in Edaravone-treated mice compared to saline-treated mice (n=12 each).
mice showed progressive reduction in the latency to find the platform, whereas saline-treated mice showed a slower decline in latency that became significant at day 4 to 5 (P=0.032 in run 1 [reference memory]; P=0.012 in run 2 [working memory] by analysis of variance; Figure 4C). On probe test, the saline-treated mice spent significantly less time in the former platform quadrant than sham animals (P=0.047 by t test). Edaravone-treated mice spent more time in the platform quadrant than saline-treated mice on probe test, but the difference was not statistically significant. These results suggest that prophylactic Edaravone treatment attenuates tHI-induced cognitive impairment.

Prophylactic, but not Delayed Application of Edaravone, Reduces tHI-Induced Infarction

Last, we compared the effects of saline (S) versus 3 regimens of Edaravone treatment in tHI-induced mortality and infarct size. In the first protocol (E1), 4.5 mg/kg Edaravone was applied at 1 hour before and 1 hour after tHI. In the second protocol (E2), 3 doses of 3 mg/kg Edaravone were injected at −1, 1, and 2 h after tHI to maintain the same total dosage. In the third protocol (E3), 2 doses of 4.5 mg/kg Edaravone were administered at 3 and 4 h after tHI to simulate delayed treatment. We recorded the mortality rate and infarct size at 24 h recovery.
This experiment showed that both E1 and E2 protocols conferred protection against tHI-induced mortality and cerebral infarct (Figure 5A). Specifically, the 24 h mortality rate in saline-treated mice was 27.6% (n=29), but dropped to 11.8% by the E1 protocol (n=17) and 16.7% by the E2 regimen (n=18; Figure 5B). In saline-treated animals (n=19), the mean infarct size was 30.5% of the contralateral hemisphere, which was reduced to 18.8% by the E2 protocol (n=14, P=0.002) and to 11.1% by the E1 protocol (n=15, P<0.0001; Figure 5C). In contrast, delayed Edaravone treatment in the E3 protocol led to a trend of higher mortality rate (38.5%, n=26) and a larger, more variable infarct size (33.8%, n=16; Figure 5B and 5C).

These results suggest that acute prophylactic administration of Edaravone within a <3 h therapeutic window reduces tHI-induced death and infarction, but delayed Edaravone treatment may be harmful.

Discussion

Stroke is a dreaded complication of cardiovascular surgery and a particular threat to individuals with symptomatic carotid artery stenosis. A high rate of silent cortical infarct also occurs after the cardiopulmonary bypass procedure. A recent study showed that 27.5% of coronary artery bypass grafting patients developed new brain infarct on diffusion-weighted imaging. Among them, 3.1% showed overt symptoms, whereas the other 24.1% were clinically silent. This high rate of silent infarct may relate to the phenomenon of postsurgery decline of cognitive functions. In terms of the pathogenesis, showers of microemboli during cardiovascular operations have been reported, and hypoperfusion is a critical mechanism linked to prolonged cardiopulmonary bypass, low cardiac output, and infarct in the watershed area. Conceivably, a long duration of cortical hypoperfusion may exacerbate the stasis of blood flow and oxidative stress in the neurovascular unit, leading to hyper-coagulability and a greater prothrombotic tendency.

Despite understanding of the pathogenesis, there are no specific drugs to prevent postsurgery stroke or cognitive deficits to date. This is because anticoagulants are unsuitable prophylactic agents because of the risk of hemorrhage. Past studies of pharmacological neuroprotectants suggested the benefits of reacemide (a NMDA receptor antagonist) and fluvastatin (a HMG-CoA reductase inhibitor) with pre- and post-surgery treatment for several days, whereas intraoperative infusion of magnesium (a multifunctional neuroprotectant) was ineffective (see Table I in the online-only Data Supplement). A recent study suggests the improvement of cognitive functions after experimental tibial fracture by perioperative stimulation of the nicotinic receptor, but whether this treatment is able to prevent postsurgery stroke is uncertain.

In the present study, we used a model of tHI to assess the effect of Edaravone as a perioperative protectant against stroke and cognitive decline. Edaravone is a lipophilic free radical scavenger that crosses blood–brain barrier and reduces post-stroke oxidative stress in multiple components of the neurovascular unit. Edaravone also possesses antiapoptosis and anti-inflammation functions in preclinical stroke models. Clinically, Edaravone is used in Japan to treat acute ischemic stroke with a 2-week, twice-a-day regimen. A recent clinical trial in Europe shows that a shorter course of higher-dose Edaravone is also safe, tolerated, and improving the neurological outcome in ischemic stroke patients (although this study was not designed to determine efficacy). Finally, the combinational tissue-type plasminogen activator–Edaravone treatment provides synergistic benefits of reduced infarct size in animal models and an increased recanalization rate for patients. Given its clinical efficacy and safety in ischemia stroke therapy, we hypothesize that Edaravone may be...
an useful prophylaxis against postsurgery stroke and cognitive decline.

The tHI model used in this study has unique features tailored for testing this hypothesis. First, unlike focal ischemia models that block terminal branches of a cerebral artery, occlusion of RCCA in the tHI model only reduces CBF to half in the ipsilateral hemisphere,9 making it closer to the extent of hypoperfusion during surgery. Second, the addition of hypoxia to hypoperfusion yields endogenous components of in-situ thrombosis, making the tHI model ideal to study the cerebral vascular bed-specific hemostasis-coagulation.31

Our experiments showed that the dual hypoxia-hypoperfusion insult triggers long-lasting reduction of CBF and oxygen saturation after the release of vascular obstruction and returning to a normoxic environment (Figures 1 and 2). These results highlight the importance of maintaining adequate blood flow and brain oxygenation for patients during surgical operations. Interestingly, when Edaravone was administered in a regimen to mimic pre- and postoperative patient care, it significantly improved cerebral reperfusion and oxygenation, while abating in-situ thrombosis (Figure 2). These physiologic effects by Edaravone are associated with marked reduction of superoxide in the brain parenchyma and the vascular wall after tHI (Figure 3). Our finding is consistent with the reports of Edaravone mitigating oxidative stress in the neurovascular unit,18–21 which may attenuate thrombosis and improve CBF recovery. Prophylactic administration of Edaravone also attenuated tHI-induced brain damage and learning deficits based on a variety of assays (Figure 4). Finally, pre- and acute post-tHI administration of Edaravone in a 2 h window reduces the mortality rate and infarct size. However, delayed Edaravone treatment after 3 h is either ineffective or harmful (Figure 5). These findings suggest that prophylactic Edaravone is an effective neuroprotectant against postsurgery stroke and cognitive decline, but future studies are needed to ensure its therapeutic window in clinical settings.

Several drugs, Edaravone included,32 have been shown to reduce infarct size if they are applied before or during experimental focal cerebral ischemia, which could signify potential to prevent postsurgery stroke. Yet, Edaravone has the unique attribute of demonstrated utility in acute ischemic stroke therapy and a favorable safety and pharmacokinetics profile. Furthermore, the use of scavengers to counter free radical-induced thrombotic tendency in the neurovascular unit carries a smaller risk of bleeding, when compared with anticoagulants. Hence, to prioritize the candidates for perioperative protection, we suggest that Edaravone merits further evaluation for potential clinical trials.

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Disclosures
Dr Abe received honoraria for speaking engagements with the Mitsubishi Tanabe Pharma Co. The other authors report no conflicts.

References


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Supplemental Table 1: Clinical trials and preclinical studies of perioperative protectants.

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<th>Study</th>
<th>Design</th>
<th>Results</th>
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<td>(Clinical trial) Neuroprotection of the brain during cardiopulmonary</td>
<td>87 subjects taking reacemide (a NMDA receptor antagonist) every 6 h from 4 days before to 5 days after cardiopulmonary bypass and 84 subjects on the placebo were compared in 9 neuropsychological tests before and 8 weeks after surgery.</td>
<td>The reacemide group showed a trend of reduction in the proportion showing decline of performance above 1 standard deviation (SD) in 2 or more testes (9% vs 12%).</td>
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<td>bypass: A randomized trial of Remacemide during coronary artery bypass</td>
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<td>in 171 patients³</td>
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<td>(Clinical trial) Fluvastatin and perioperative events in patients</td>
<td>250 subjects taking fluvastatin from randomization to at least 30 days after vascular surgery and 247 subjects on the placebo were compared for the onset of myocardial ischemia in 30 days post-operation. The secondary end-point was composite death from cardiovascular causes and myocardial infarction.</td>
<td>The fluvastatin group showed fewer myocardial ischemia (10.8% vs 19%) and fewer deaths from myocardial infarction and cardiovascular causes (4.8% vs 10.1%).</td>
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<td>(Clinical trial) Intraoperative magnesium treatment does not improve</td>
<td>198 subjects with magnesium infusion and 191 subjects with the placebo during cardiopulmonary bypass were assessed for cognitive functions pre-operatively and again at 6 weeks postoperatively.</td>
<td>The incidence of cognitive deficit in the magnesium group was 44.4% compared with 44.9% in the placebo group. Magnesium therapy did not improve neurocognitive function after cardiac surgery.</td>
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<td>neurocognitive function after cardiac surgery³</td>
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<td>(Preclinical study) Resolving postoperative neuroinflammation and</td>
<td>278B/6J underwent stabilized tibial fracture were assessed by trace fear conditioning (TFC) for memory functions. Agonists and antagonists of the nicotinic acetylcholine receptor (nAChR) were given to the mice prior to TFC testing.</td>
<td>Tibial fracture decreased freezing behavior, the index for memory retention in the TFC test. Memory deficits were worsened by antagonists of nAChR but prevented by the administration of nAChR agonists.</td>
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<td>cognitive decline⁴</td>
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