Attenuation of Cerebral Vasospasm After Subarachnoid Hemorrhage in Mice Overexpressing Extracellular Superoxide Dismutase

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Background and Purpose—Subarachnoid hemorrhage (SAH) increases production of vascular extracellular superoxide anion (\(\text{O}_2^-\)). We examined whether overexpression of murine extracellular superoxide dismutase (EC-SOD) alters SAH-induced cerebral vasospasm, oxidative stress, and neurological outcome.

Methods—Mice exhibiting a 2-fold increase in vascular EC-SOD and wild-type (WT) littermates were subjected to sham surgery or SAH by perforation of the right anterior cerebral artery. Neurological deficits were scored 72 hours later. Middle cerebral artery (MCA) diameter was measured or immunohistochemically stained for nitrotyrosine.

Results—MCA diameter (mean±SD) was greater in EC-SOD versus WT mice after SAH but not sham surgery (EC-SOD SAH=56±10 \(\mu\)m; WT SAH=38±13 \(\mu\)m \(P<0.01\); EC-SOD sham=99±16 \(\mu\)m; WT sham=100±15 \(\mu\)m). SAH decreased median (range) neurological score (scoring scale, 9 to 39; no deficit=39) versus shams, but there was no difference between EC-SOD and WT groups (EC-SOD SAH=26 [23 to 30]; WT SAH=23 [19 to 29] \(P=0.27\); EC-SOD sham=39 [39]; WT sham=39 [39]). Sensory-motor deficits correlated with MCA diameter \((P<0.001)\) but worsened primarily between 60 and 50 \(\mu\)m, plateauing below this threshold. The percentage of mice with MCA nitrotyrosine staining increased after SAH in WT (sham=29%; SAH=100% \(P<0.05\)) but not EC-SOD (sham=33%; SAH=44% \(P=0.80\)) mice.

Conclusions—Endogenous overexpression of EC-SOD attenuated vasospasm and oxidative stress but failed to reduce neurological deficits after SAH. Extracellular \(\text{O}_2^-\) likely plays a direct role in the etiology of vasospasm. (Stroke. 2002; 33:2317-2323.)

Key Words: extracellular space • mice • mice, transgenic • subarachnoid hemorrhage • superoxide dismutase • vasospasm

Cerebral vasospasm is a chronic, delayed arterial intraluminal narrowing that may occur after aneurysmal subarachnoid hemorrhage (SAH).1 Although vasospasm remains a major cause of cerebral ischemic mortality and morbidity, the pathogenesis of vasospasm remains incompletely understood.

There is evidence that superoxide anion (\(\text{O}_2^-\)) production is enhanced by SAH.2 Vasospasm may be attributable in part to effects of hemorrhage on interactions between \(\text{O}_2^-\) and nitric oxide (NO). Both a decrease in NO-mediated vasodilation and augmentation of protein kinase C–dependent contraction have been demonstrated after SAH.3–5 Reactive oxygen species stimulate both pathways. \(\text{O}_2^-\) reacts with NO to form peroxynitrite (ONOO\(^-\)), potentially depleting NO available for vasodilation. Superoxide has also been proposed to produce direct vasoconstriction, although this is controversial.6

Superoxide dismutase (SOD) converts \(\text{O}_2^-\) to \(\text{H}_2\text{O}_2\) and may attenuate vasospasm after SAH. The efficacy of SOD in preventing vasospasm has varied among studies. Intracisternal injection of SOD was effective in reducing the magnitude of vasospasm induced by subarachnoid injection of autologous blood in both cat and rabbit models.7,8 This effect was not observed with intrathecal injection of both catalase and SOD in monkeys.9 This discrepancy may be a result of bioavailability of SOD or timing of treatment. Transgenic models of endogenous SOD overexpression eliminate complicating issues of half-life, bioavailability, and routes of administration for exogenous SOD. Recently, transgenic mice overexpressing CuZn-SOD were found to have partial resolution of vasospasm when examined 3 days after SAH.10 Whether exogenous SOD or upregulation of endogenous SOD can reduce neurological injury from vasospasm has not been investigated.
Mammalian species have 3 SOD isoenzymes. CuZn-SOD and Mn-SOD are principally located in the cytosol and mitochondria, respectively. Thus, the importance of \( \cdot O_2^- \) localized in the extracellular compartment remains undefined by study of mice overexpressing CuZn-SOD. Greater than 90% of extracellular SOD (EC-SOD) is found in the extracellular space.\(^{11,12}\) EC-SOD is highly expressed in the vascular endothelium and contributes up to 70% of total SOD activity in arteries.\(^{13,14}\) Immunolocalization studies have demonstrated that EC-SOD is located in the arterial intimal extracellular matrix.\(^{13,14}\) NO traverses this matrix to stimulate smooth muscle relaxation. Therefore, extracellular \( \cdot O_2^- \) may have an important role in modulating SAH-induced vasospasm. Study of EC-SOD can allow unique examination of the contribution of extracellular \( \cdot O_2^- \) to the pathogenesis of vasospasm. We hypothesized that transgenic overexpression of EC-SOD would ameliorate vasospasm resultant from SAH in the mouse. We also examined whether this effect would be demonstrated that EC-SOD is located in the arterial intimal extracellular matrix.\(^{13,14}\) NO traverses this matrix to stimulate smooth muscle relaxation. Therefore, extracellular \( \cdot O_2^- \) may have an important role in modulating SAH-induced vasospasm. Study of EC-SOD can allow unique examination of the contribution of extracellular \( \cdot O_2^- \) to the pathogenesis of vasospasm. We hypothesized that transgenic overexpression of EC-SOD would ameliorate vasospasm resultant from SAH in the mouse. We also examined whether this effect would be sufficient to alter neurological injury from vasospasm and whether EC-SOD overexpression could be associated with evidence of reduced oxidative stress as defined by immunohistochemical staining for vascular nitrotyrosine.

Materials and Methods

Transgenic Mice

All experiments were approved by our Animal Care and Use Committee. Male EC-SOD transgenic mice used for this experiment have been described previously.\(^{15,16}\) Briefly, EC-SOD transgenic mice were generated by microinjecting nuclei of fertilized oocytes from (C57BL/6×C3H)F1 female mice with the cDNA of human EC-SOD containing a \( \beta \)-actin promote. Mice carrying the transgene were identified by polymerase chain reaction (PCR) amplification of the human EC-SOD gene and back-bred into (C57BL/6×C3H)F1 mice. Brain parenchymal EC-SOD activity in these transgenic mice has previously been shown by different techniques to be increased 5- to 10-fold compared with wild-type littermates.\(^{15,16}\) The activities of the other antioxidant enzymes were unchanged. Effects of the transgene on magnitude of vascular EC-SOD activity have not been defined previously (see below).

All mice were 10 to 12 weeks of age. Age- and sex-matched littermates not containing the EC-SOD transgene, as defined by PCR analysis on tissue from each animal, served as wild-type cohorts.

Definition of Vascular EC-SOD

Reverse Transcription–PCR

Aortas were dissected from EC-SOD transgenic mice and wild-type littermates, and RNA was isolated with the use of acid phenol–chloroform extractions as previously described.\(^{16,18}\) Message for human EC-SOD or mouse EC-SOD was detected by reverse transcription–polymerase chain reaction (RT-PCR) with the use of a Gene-ampliRTh PCR kit (Perkin Elmer) and primers specific for either mouse EC-SOD (forward, 5’-TTCTGTGTTCTACGGCTT-GCTAC-3’; reverse, 5’-CTCCATCCAGATCTCCAGCACT-3’) or human EC-SOD (forward, 5’-AGAACCCTTCCACTGAGG-3’; reverse, 5’-GTTCGCTTACAATGTGAGGC-3’). PCR products were detected after agarose gel electrophoresis by ethidium bromide staining. As an additional control, samples were pretreated with RNase before RT-PCR to ensure that the product was specific to EC-SOD mRNA and not due to DNA contamination.

Analysis of Vascular EC-SOD Activity

Aortas from 5 EC-SOD transgenic mice and 11 wild-type littermates were pooled separately for analysis of EC-SOD activity. The aortas were homogenized in 3 mL of 50 mmol/L potassium phosphate, 0.3 mol/L potassium bromide, pH 7.4. After separation from CuZn-SOD and Mn-SOD by concanavalin A–sepharose chromatography,\(^{19-21}\) EC-SOD activity was measured by inhibition of partially acylated cysteine cytochrome C reduction at pH 10.0, as previously described.\(^{22}\) The total protein concentration in the homogenates was determined by the Coomassie Plus protein assay (Pierce).

SAH Model

All animals were subjected to fasting for 12 hours to control plasma glucose concentration. Anesthesia was induced in a chamber with 5% halothane in 50% \( O_2 \)/45% \( N_2 \). The trachea was intubated, and the lungs were mechanically ventilated. Pericranial temperature was maintained at 37.0±0.5°C with the use of a heat lamp and a pericranial needle thermometer. Anesthesia was maintained with 1.0% halothane in 50% \( O_2 \)/49% \( N_2 \).

EC-SOD transgenic and wild-type littermates were randomly assigned to undergo SAH or sham surgery. The right common carotid artery was exposed by a midline incision of the neck, and the external carotid artery (ECA) was isolated and ligated. A blunt 5-0 monofilament nylon suture was introduced into the ECA and advanced into the internal carotid artery (ICA). The suture was advanced distal to the right anterior cerebral artery (ACA)–middle cerebral artery (MCA) bifurcation, where resistance was encountered, and then advanced 3 mm further to perforate the right ACA. The suture was immediately withdrawn through the ICA into the ECA, allowing reperfusion and SAH. In sham-operated mice the suture was advanced only until the point of resistance, thereby avoiding arterial perforation. After removal of the filament, the skin was closed with suture. Halothane was discontinued. On recovery of spontaneous ventilation, the trachea was extubated. Mice were continuously observed until recovery of the righting reflex and were then returned to their cages. Subcutaneous injections of 10% dextrose in 0.9% NaCl (0.5 mL) were given twice per day to all mice to standardize hydration. All surgeries were performed by the same surgeon, who was blinded to mouse genotype.

To obtain an estimate of physiological status during the SAH insult, 5 transgenic mice and 5 wild-type mice underwent anesthesia and surgical preparation for SAH surgery as described above. In addition, a catheter was placed in the left femoral artery. Mean arterial blood pressure, \( pH \), \( P_{acO_2} \), and \( P_{atO_2} \) were measured immediately before and 20 minutes after SAH.

Neurological Evaluation

A neurobehavioral examination (scoring scale, 9 to 39) was performed on each recovery animal at 72 hours after SAH or sham surgery. This examination was adapted from 2 previously described examinations in which the scoring systems were combined.\(^{23,24}\) A motor score (0 to 12) was derived from spontaneous activity, symmetry of limb movements, climbing, balance, and coordination. A sensory score (5 to 15) was derived from body proprioception, vibrissae, visual, olfactory, and tactile responses. A reflex score (4 to 12) was derived from righting, postural, ear, and eye reflexes.

Cerebral Vascular Perfusion

Ten wild-type sham, 8 wild-type SAH, 10 EC-SOD sham, and 10 EC-SOD SAH mice underwent cerebral vascular perfusion 72 hours after surgery, when vasospasm has been reported to peak in this model.\(^{10}\) Mice were anesthetized with halothane. The tracheas were intubated, and the lungs were mechanically ventilated. The chest was opened, and the aorta was cannulated with a blunt 20-gauge needle through the left ventricle. Flexible plastic tubing (3.2-mm internal diameter) connected to the 20-gauge needle was used to deliver infusion solutions by manual pulsatile syringe pressure. The tubing was connected to a 30-mL syringe, the cannulated aorta, and a mercury manometer, establishing a closed circuit to monitor perfusion pressure. An incision was made in the right atrium for outflow of perfusion solutions. Twenty milliliters of normal saline was infused, followed by 15 minutes of 4% formalin infusion and 10 minutes of gelatin–india ink solution. All perfusates were passed through a 0.2-µm (pore size) filter and delivered at a pressure of 80 to 60 mm Hg. Prior work has shown that these procedures markedly
reduce fixation artifacts.25 The mouse was then refrigerated for 24 hours to allow gelatin solidification. The brains were harvested and stored in 4% neutral buffered formalin.

Cerebrovascular casting methods have previously been used to assess vasospasm in large arteries as well as to study architecture of cerebral microvessels.10,26 Furthermore, perfusion fixation has become standard in the histological measurement of lumen diameters in rat and rabbit SAH models.27,28

Analysis of Hemorrhage

Brains were analyzed under light microscopy to determine the magnitude of SAH by an observer blinded to experimental group. Hemorrhage size was graded by 2 characteristics: area of hemorrhage distribution and density of clot formation. Hemorrhage size was scored as follows: 1, SAH extends anteriorly <1000 μm from MCA-ACA bifurcation; 2, SAH extends >1000 μm anteriorly from bifurcation; and 3, SAH extends >1000 μm anteriorly from bifurcation with posterior extension across the ICA. Hemorrhage density was scored as follows: 1, underlying brain parenchyma visualized through clot; and 2, underlying brain parenchyma not visualized through clot. Hemorrhage grade (2 to 5) was determined by the sum of the size and density scores. Absence of hemorrhage was scored as 0.

Vascular Diameter Measurement

Blood vessels were imaged with the use of a video-linked dissecting microscope controlled by an image analyzer (MCID-M2 3.0, Imaging Research Inc). The image of each section was stored as a 1280×960 matrix of calibrated pixel units and displayed on a video screen. Images of the MCA were divided into a proximal 1000-μm and distal 1000-μm segment from the ACA-MCA bifurcation. Because vasospasm occurred most prominently near the center of hemorrhage and visualization of the proximal ACA was often distorted by clot formation in our pilot studies, the proximal MCA segment was recorded by digital measurement. To attribute differences in MCA diameters to local vasospasm and not variations in gelatin-ink perfusion, the basilar artery diameter was measured in all SAH mice. A single observer blinded to experimental group performed all vascular measurements.

Nitrotyrosine Immunohistochemistry

A subset of mice was randomly used to assess MCA protein nitrosylation. Three days after sham or SAH surgery, mice were perfused in situ with 4% paraformaldehyde in PBS, pH 7.4. Brains were paraffin embedded, and 5-μm cross sections of the right MCA were cut and fixed to slides. In all mice, cross sections of the MCA were sampled at approximately 500 μm distal to the MCA-ICA bifurcation. In our pilot studies, this point was selected as the primary end point to determine magnitude of vasospasm. The smallest lumen diameter within the proximal MCA segment was recorded by digital measurement. To attribute differences in MCA diameters to local vasospasm and not variations in gelatin-ink perfusion, the basilar artery diameter was measured in all SAH mice. A single observer blinded to experimental group performed all vascular measurements.

Sections were examined by a blinded observer for the presence of nitrotyrosine staining of the MCA vascular tissues. To confirm specificity of the primary and secondary antibodies, sections were incubated without the primary antibody or with the primary antibody in the presence of 10 mmol/L 3-nitro-l-tyrosine (Cayman Chemical).

Statistical Analysis

Continuous data (eg, vascular diameters, physiological values) were compared by 1-way ANOVA. In the case of a significant F ratio, post hoc analysis was performed with the protected least squares difference test (StatView 5.0, SAS Institute). The α error was set at 0.05. Nonparametric data (eg, neurological scores, SAH grade) were compared with the Kruskal-Wallis H statistic. The Mann-Whitney test was used for intergroup comparisons. Frequency of nitrotyrosine immunohistochemistry staining was compared by 2-tailed Fisher exact test. Correlations between neurological score, MCA diameter, and SAH grade were analyzed with the Spearman rank correlation coefficient. Parametric values are given as mean±SD. Nonparametric values are given as median (interquartile range [IQR]).

Results

Analysis of Vascular EC-SOD

RT-PCR analysis of RNA from aortas of EC-SOD transgenic and wild-type littermates showed positive message for mouse EC-SOD in both the transgenic and wild-type animals. In contrast, message for the human EC-SOD transgene was only present in the EC-SOD transgenic mice, indicating that the transgene was actively transcribed into the aortas of the transgenic animals. Pretreatment of the samples with RNAse resulted in no amplification, confirming that the results were not due to DNA contamination. Direct measurement of EC-SOD activity in the aortas further confirmed that the EC-SOD transgene was being expressed in the aortas of the transgenic animals. The transgenic mice had a 2-fold increase in EC-SOD activity compared with the wild-type littermates (transgenic=3.2 U/mg protein, pH 10; wild-type=1.6 U/mg protein, pH 10).

Physiological Values

There were no differences in PaCO2 (EC-SOD=40±4 mm Hg; wild-type=40±4 mm Hg), Pao2 (EC-SOD=171±16 mm Hg; wild-type=181±11 mm Hg), temperature (EC-SOD=37.0±0.01°C; wild-type=37.0±0.01°C), or mean arterial blood pressure (EC-SOD=69±2 mm Hg; wild-type=72±3 mm Hg) between surrogate transgenic and wild-type mice during SAH surgery. Body weight did not differ between transgenic (25±2 g) and wild-type (25±2 g) groups. SAH was distributed in the basal cistern of the brain of all SAH mice. The mortality rate within 72 hours was 9% (5 transgenic and 3 wild-type mice died). In each of these mice, severe SAH extending bilaterally was evident at necropsy.

Changes in Proximal MCA Lumen Diameter

In mice used for MCA diameter analysis, the SAH grade in wild-type (4 [IQR, 3 to 4]; n=8) and transgenic mice (4 [IQR, 3 to 5]; n=10) was not different between groups (P=0.18).

In wild-type mice, SAH caused a 62% reduction in mean MCA diameter (SAH=38±13 μm; n=8; sham=100±15 μm, n=10; P<0.001). In transgenic mice, SAH caused a 43% reduction in mean MCA diameter (SAH=56±10 μm, n=10).
The MCA lumen diameter was larger in transgenic (TG) versus wild-type (WT) mice (P=0.0001). MCA diameter was reduced in both SAH groups. Vessel diameter after SAH was greater in EC-SOD present (P=0.0001). MCA diameter was reduced in both SAH groups. A main effect for treatment group was present (P=0.0001).

Neurological Function
In mice used for neurological analysis, SAH grades in wild-type (3 [IQR, 2 to 4]) and transgenic mice (3 [IQR, 2 to 4]) were similar (P=0.64).

In wild-type mice, neurological score was worsened 3 days after SAH (23 [IQR, 19 to 29]) versus wild-type shams (39 [IQR, 39]; P<0.005). In transgenic SAH mice, neurological score was also worsened (26, [IQR, 23 to 30]) versus transgenic shams (39 [IQR, 39]; P<0.005). Neurological scores, however, were not different between transgenic and wild-type SAH mice (P=0.20) (Table).

Nitrotyrosine
For nitrotyrosine analysis, SAH grade was 3 or 4 in all mice. The percentage of mice with positive MCA nitrotyrosine immunolabeling was increased in wild-type SAH (100%, n=8) versus wild-type sham mice (29%, n=7; P<0.01). There was no difference in percentage of mice with positive nitrotyrosine immunolabeling in transgenic SAH (44%, n=9) versus transgenic sham mice (33%, n=6; P=0.80). Between SAH groups, fewer transgenic mice had positive immunolabeling (44%, n=9) than wild-type mice (100%, n=8; P<0.05) (Figure 4).

Discussion
In this study overexpression of EC-SOD reduced morphological vasospasm as measured 3 days after SAH. Although both wild-type and transgenic mice displayed vasospasm, a 62% reduction in vascular diameter was observed in wild-type mice compared with a 43% reduction in transgenic mice. Physiological values, MCA vascular diameter, and neurological function after sham surgery were similar in the transgenic and wild-type groups, indicating similar phenotypic baselines on which the effects of SAH were superimposed. Similarities of basilar sensory performance correlated with SAH grade (P<0.001) and with MCA diameter (P<0.001; n=38) (Figure 3).
artery diameters and SAH grades between transgenic and wild-type mice indicate that an intergroup difference in gelatin-ink perfusion or hemorrhage magnitude did not contribute to the transgenic effect on MCA diameter. Neurological deficits were most likely attributable to vasospasm on the basis of both a correlation with MCA diameter and localization of behavioral deficits to the MCA vascular distribution. Although a 30% attenuation of vasospasm was observed with EC-SOD overexpression, this effect did not result in an improved neurological outcome. An increase in nitrotyrosine immunostaining was observed in wild-type mice after SAH, suggesting that oxidative stress and peroxynitrite formation occurred. The reduction in nitrotyrosine immunostaining after SAH in EC-SOD mice indicates that EC-SOD attenuation of oxidative stress, at least in part, explains the effect observed.

Endovascular perforation of cerebral arteries in rats and mice produces SAH without craniotomy or other invasive techniques that may confound in vivo responses. Genetically engineered mice allow for increased levels of singular enzymatic activity, eliminating complicating issues of half-life, bioavailability, and routes of drug administration associated with exogenously administered compounds. At the same time, although prior work with these mice has shown no changes in the activities of other antioxidant enzymes, we cannot be sure that unrecognized ontogenetic adaptation to EC-SOD overexpression did not contribute to the results observed. Furthermore, because of the relatively small extracellular compartment and amount of EC-SOD in brain, sufficiently sensitive EC-SOD antibodies have not been developed to confirm localization of EC-SOD overexpression in the cerebrovascular extracellular compartment. Extracellular localization of EC-SOD has been documented clearly in the lung and aorta.

Severities of vasospasm have been shown to be dependent on magnitude of SAH. To attribute differences in vasospasm solely to EC-SOD overexpression, we developed a SAH grading scale allowing us to account for SAH variability. The degree of vasospasm was proportional to both density and size of SAH in our pilot studies, suggesting that measurement of both variables would provide a reliable grading scale. In this study there were no differences between SAH grades in EC-SOD overexpressors versus wild-type mice. As a result, further evidence is provided that differences in vascular diameter responses to SAH were the result of the EC-SOD genotype.

The formation of nitrotyrosine in the vasospastic MCA further suggests that NO degradation to peroxynitrite and subsequent oxidative stress play a role in cerebral vasospasm. Nitrotyrosine staining was observed primarily at the sites of NO production, the adventitia, and endothelium. The failure of EC-SOD overexpression to completely resolve vasospasm in this study may be a result of overwhelming \( \cdot O_2^- \) formation after SAH. This is supported by the persistence of nitrotyrosine formation in some EC-SOD overexpressors after SAH. The 2-fold increase in vascular EC-SOD activity in these transgenic mice most likely was insufficient to completely prevent oxidative disruption of vascular tone regulation. This is consistent with the findings of Kamii et al, who compared CuZn-SOD overexpressors to their wild-type counterparts in a mouse SAH model. Although the magnitude of change in CuZn-SOD activity was not reported, CuZn-SOD overexpression resulted in a 50% reduction of SAH-induced vasospasm. Kamii et al observed MCA diameter reduction of only 20% with wild-type vasospasm compared with 62% in our model, prohibiting direct comparison of magnitudes of effect between the 2 SOD isozymes. Because CuZn-SOD and EC-SOD are localized in different compartments, however, evidence is now available that \( \cdot O_2^- \) present in both intracellular and extracellular compartments contributes to the pathogenesis of vasospasm. Thus, pharmacological mimics of the respective SOD isozymes may have additive effects. There also is current interest in use of intrathecal gene transfer technology to use EC-SOD as a therapeutic tool in SAH-induced cerebral vasospasm.

Although exogenous SOD and CuZn-SOD have been shown to partially resolve vasospasm after SAH, the impor-
tance of this effect on neurological damage has not previously been measured.7,10 In our study motor and sensory deficits correlated with the magnitude of MCA vasospasm and were anatomically localized predominantly in the MCA vascular distribution. Reflex function was only modestly affected. Mean vision deficit was half that of other sensory tasks. These findings suggest that the measured neurological deficits resulted from MCA vasospasm and resultant hypoperfusion of its vascular bed. Our findings indicate that a threshold phenomenon of ischemia and resultant neurological deficits occurred between MCA diameters of 60 and 50 µm (Figure 3). Motor and sensory deficits began to worsen below MCA diameters of 60 µm. Deficits did not worsen below MCA diameter of 50 µm. This may explain why improvement of mean MCA diameter from 38 to 56 µm did not significantly improve neurological deficits in the EC-SOD overexpressors. If so, we would predict that other therapies, administered in combination with EC-SOD overexpression, or even greater EC-SOD expression alone, may provide sufficient advantage to allow demonstration of improved neurological function.

Since the initial description of free radical generation by clot lysis in SAH,34 multiple studies have indicated that lipid peroxidation and reactive oxygen species formation play a role in the pathogenesis of vasospasm.43–45 The breakdown of oxyhemoglobin to methemoglobin is thought to release $\cdot O_2^-$,36 which rapidly reacts with NO to produce a more potent species, peroxynitrite.37 Nitrotyrosine, a byproduct of peroxynitrite interaction with cellular proteins, was shown to increase concurrently with vasospasm after SAH, supporting the concept of a "NO sink" phenomenon in the etiology of vasospasm,46 shifting the balance of the vascular tone toward vasoconstriction. These previous reports are consistent with the attenuation of vasospasm and nitrotyrosine formation observed with EC-SOD overexpression in this study.

In conclusion, a 2-fold increase in vascular EC-SOD activity caused attenuation of both cerebral vasospasm and vascular nitrotyrosine formation after SAH. The magnitude of this effect was below the threshold necessary to improve neurological deficits attributable to vasospasm. Nevertheless, the data are consistent with numerous reports that either endogenous or exogenous enhancements of superoxide dismutase can, in part, ameliorate vasospastic responses to SAH and that combined therapies that include dismutation of $\cdot O_2^-$ in the extracellular compartment may be worth pursuing.

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