

## Chapter 49

# Simvastatin Attenuates Experimental Cerebral Vasospasm and Ameliorates Serum Markers of Neuronal and Endothelial Injury in Patients After Subarachnoid Hemorrhage: A Dose-Response Effect Dependent on Endothelial Nitric Oxide Synthase

Matthew J. McGirt, M.D., Graeme F. Woodworth, B.S., Gustavo Pradilla, M.D., Federico Legnani, M.D., David Warner, M.D., Rafael Tamargo, M.D., Richard Clatterbuck, M.D., John R. Lynch, M.D., and Daniel T. Laskowitz, M.D.

Delayed cerebral ischemic injury secondary to vasospasm is a major cause of morbidity and mortality after subarachnoid hemorrhage (SAH) (28). Currently, there are no medical treatments that consistently prevent or reverse cerebral vasospasm. Recent studies suggest vasospasm is the result of a multifactorial process leading to a functional imbalance in the cerebrovascular smooth muscle tone (9, 10). Two potential etiologies include microvascular inflammatory cell infiltration and endothelial cell damage, both contributing to a subsequent deficiency of nitric oxide (NO) (6, 7).

Numerous studies have begun to elucidate the role of the inflammatory response to SAH in the pathogenesis of cerebral vasospasm (11, 45). Leukocyte function antigen-1 (LFA-1) binding to endothelial intracellular adhesion molecules (ICAMs) is required to initiate leukocyte migration through the intima and subsequent migration into the central nervous system (20), a process that is potentiated by SAH and plays a central role in the development of cerebral vasospasm (14, 36). Perivascular chemokine activated leukocytes synthesize and release endothelin-1, a potent vasoconstrictor, as well as superoxide free radicals, leading to endothelial damage and decreased endothelial production of NO (12, 30).

The vascular endothelium is known to regulate vascular smooth muscle tone by generating NO (25) and other endothelial-derived signaling molecules (50). NO synthase (NOS), the primary source of endothelium-derived NO, use L-arginine to form NO and citrulline. The endothelial isoform (eNOS) is constitutively expressed in cerebrovascular endothelium and is essential for NO-dependent vascular tone (18). eNOS protein, eNOS messenger (m)RNA, and eNOS immunoreactivity have been shown to decrease after SAH (24, 42). Pharmacological NO replacement has been reported to reverse cerebral vasospasm in animal studies (41, 49).

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, known as statins, are potent inhibitors of cholesterol biosynthesis (19). Recent studies demonstrate that treatment with statins improves endothelial function without changes in serum cholesterol (38, 48). Additionally, numerous studies have shown statins have diverse antiinflammatory and cell-signaling effects (8, 43). Statins have been shown to decrease ICAM-1 expression and competitively inhibit the LFA-1–ICAM interaction (5, 47). Statins also interfere with multiple steps of leukocyte recruitment and migration by inhibiting monocyte and endothelial production of interleukins, chemokines, and matrix metalloproteinase-9 (4, 16). Statins have also been shown to directly upregulate eNOS expression under cholesterol-controlled conditions (31, 33). A threefold increase in eNOS mRNA, protein, and enzymatic activity has been demonstrated after statin treatment, resulting in an increase in cerebral blood flow (2, 13). Selective up-regulation of eNOS activity with statin treatment may prevent eNOS depletion or even increase eNOS expression after SAH.

We examined if administration of simvastatin before SAH increases endogenous eNOS protein, attenuates cerebral

vasospasm, or reduces delayed cerebral ischemic injury in wild-type and eNOS-deficient mice. Next, we explored if the administration of simvastatin after the onset of SAH attenuates cerebral vasospasm in rabbits. We then examined if simvastatin attenuates experimental cerebral vasospasm at clinically approved doses and is safe in patients with SAH.

## METHODS

### Pre-SAH Treatment Studies

All experiments were approved by the Duke University or Johns Hopkins University Animal Care and Use Committee. Simvastatin (Zocor, Merck and Co., Inc., West Point, PA) was chemically activated by alkaline hydrolysis, as previously described (17, 31). C57BL6/J male mice (The Jackson Laboratory, Bar Harbor, ME; 10–12 wk old) were subcutaneously injected once daily with simvastatin or a corresponding volume of vehicle for 14 days. Large-dose group = 38 mice received simvastatin 20 mg/kg or a vehicle before SAH or sham surgery. Motor function was assessed for 3 days after SAH by Rotorod (RR) testing. Middle cerebral artery (MCA) diameter and cerebrovascular eNOS protein were measured 72 hours after SAH. The study was repeated in 16 wild-type versus transgenic eNOS-knockout mice. Small-dose group = 30 mice received simvastatin 1 mg/kg or 0.2 mg/kg or a vehicle before SAH or sham surgery, and motor function was assessed for 3 days after SAH.

### Murine SAH Model

All surgeries were performed by the same surgeon who was blinded to drug treatment group. Mice were fasted from food for 12 hours before surgery to control plasma glucose concentration. Anesthesia was induced in a chamber with 5% halothane in 50% O<sub>2</sub>/45% N<sub>2</sub>. The trachea was intubated, and the lungs were mechanically ventilated. Anesthesia was maintained with 0.8 to 1.0% halothane in 50% O<sub>2</sub>/balance N<sub>2</sub>. Simvastatin- or vehicle-treated mice were randomly assigned to undergo SAH or sham surgery. The right common carotid was exposed by a midline incision of the neck, and the external carotid artery (ECA) was isolated and ligated. A blunted 5-0 monofilament nylon suture, was introduced into the ECA and advanced into the internal carotid artery. The suture was advanced distal to the right anterior cerebral artery (ACA)-MCA bifurcation, where resistance was encountered, and then advanced 3 mm further to perforate the right ACA. The suture was immediately withdrawn, 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, and halothane was discontinued. Upon 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 for 3 days. Daily simvastatin and vehicle injections were continued throughout this recovery interval.

### Murine Neurological Evaluation

The RR test was used to assess motor coordination and balance (22). Briefly, mice were held by their tails and placed on the RR facing the wall by using gentle swinging motion. The protocol included a conditioning session before injury and a testing latency time session daily for 3 days after SAH. For conditioning, mice were placed on the RR at the lowest constant rotary speed (lock mode) for 60 seconds. This was done twice, with the interval between trials being 5 minutes. For latency time testing, mice were placed on the RR, which was accelerated at a constant

rate for all mice. The latency time to either falling from the rod or turning 360 degrees twice (stationary and holding on to the rod) was recorded. The mean latency time for the two trials was reported.

#### Murine Cerebrovascular Perfusion

Cerebral vascular perfusion was performed by an observer blinded to group assignment 72 hours after surgery, when vasospasm has been reported to peak in this model (27). 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 blunted 23-gauge needle via the left ventricle. Flexible plastic tubing (3.2-mm internal diameter) connected to the 23-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 to allow for outflow of perfusion solutions. Twenty milliliters of 0.9% NaCl was infused, followed by 15 minutes of 10% formalin infusion and 10 minutes of gelatin-India ink solution. All perfusates were passed through a 0.2- $\mu$ m pore size filter and delivered at 60 to 80 mm Hg (21). The mouse was then refrigerated for 24 hours to allow gelatin solidification. The brains were harvested and stored in 4% neutral buffer formaldehyde. This cerebrovascular casting method has previously been used to assess vasospasm in large arteries, as well as to study architecture of cerebral microvessels (26, 27). Furthermore, perfusion fixation has become standard in the histological measurement of lumen diameters in rat and rabbit SAH models (3, 39).

#### Murine Vascular Diameter Measurement

Blood vessels were imaged using a video-linked dissecting microscope (6 $\times$  magnification) controlled by an image analyzer (MCID-M5; Imaging Research Inc., St. Catharines, Ontario, Canada). The image of each section was stored as a 1280  $\times$  960 matrix of calibrated pixel units and displayed on a video screen. Images of the MCA were divided into a proximal 1.0-mm and distal 1.0-mm 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 was selected as our 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 recorded all vascular measurements.

#### Cerebrovascular eNOS Immunoblot Analysis

Mice not undergoing cerebrovascular casting underwent eNOS Western blot analysis immediately after neurological examination 3 days after SAH or sham surgery. Brain tissue ipsilateral to SAH or sham surgery, within the vascular distribution of the MCA, was rapidly harvested after halothane-induced euthanasia and frozen in liquid nitrogen. All samples were stored at  $-80^{\circ}\text{C}$  until use.

For immunoblot analysis, tissue was placed into 0.3 to 0.4 mL of extraction buffer (45 mM of tris-HCl; 0.4% sodium dodecyl sulfate; 0.2 mM of phenylmethylsulfonyl fluoride; 1 mM of sodium vanadate; and 0.5% Sigma protease inhibitor cocktail), sonicated, and centrifuged for 5 min at 12,000 rpm. A portion of the supernatant was used for total protein analysis, and the remainder combined with a Western blot loading buffer. Ten micrograms of protein (diluted

in buffer) was loaded onto each lane of the gel (7.5% SDS-PAGE gel with 5% stacking gel and Tris-glycine running buffer). Each gel was presented with two samples each from group, along with a standard (human umbilical vein endothelial cell extract; Transduction Laboratories, Lexington, KY). To confirm that each lane contained equivalent amounts of protein, one duplicate gel was run in parallel and stained for total protein using Gelcode Blue. The original gel was wet-transferred to a polyvinylidene fluoride (PVDF) membrane using CAPS/methanol buffer. The membranes were blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS)/Tween 20. This was followed by overnight incubation at 4 °C with rabbit-anti-eNOS antibody (Transduction Laboratories). After PBS/Tween rinsing, the specimens underwent a 2-hour incubation with the secondary antibody (goat anti-rabbit immunoglobulin G: horseradish peroxidase) and rinsed again in PBS/Tween. For quantitation of eNOS expression, bands were visualized by incubation in enhanced chemiluminescence reagents and exposure to x-ray film. Quantitative assessment of band intensities (integrated optical densities) was performed by an observer blinded to experiment group using an Alpha Innotech Imaging 2000 system (Temucula, CA). Background intensities (determined from an equal-sized area of the film adjacent to the band of interest) were subtracted.

## Post-SAH Treatment Studies

### Rabbit Study

Fifteen New Zealand white rabbits (*Oryctolagus cuniculus*) (Robinson Co., Winston Salem, NC) weighing 1.5 to 2.5 kg were subcutaneously injected once daily 30 minutes, 24 hours, and 48 hours after sham or SAH surgery with simvastatin (40 mg/kg, 5 mL) or a corresponding volume of vehicle. Fifteen rabbits were randomized to three groups: vehicle-sham (n = 5), vehicle-SAH (n = 5), and simvastatin-SAH, (n = 5).

### Rabbit SAH Model

We have previously described the surgical technique for the rabbit SAH model (15). Briefly, rabbits were anesthetized by intramuscular injection of a mixture of ketamine (50 mg/kg) (100 mg/mL; Ketamine HCL; Abbot Laboratories, Chicago, IL) and xylazine (10 mg/kg) (100 mg/mL; Xyla-ject; Phoenix Pharmaceutical, St. Joseph, MO). After the induction of anesthesia, ceftriaxone IM (20 mg/kg) was administered, and through a midline incision, the atlantooccipital membrane was exposed. Rabbits in the sham group were irrigated with saline solution and closed. In the other two experimental groups (SAH-vehicle and SAH-simvastatin), 1 mL of cerebrospinal fluid was aspirated by cisternal puncture. Nonheparinized blood from the central ear artery (1.5 mL) was then injected into the cisterna magna. After 30 minutes in the head-down position, the rabbits were allowed to recover.

### Basilar Artery Lumen Measurement

Peak vasospasm in the rabbit model of SAH is present at 72 hours after blood injection into the cisterna magna. All rabbits were euthanized at 72 hours after the induction of experimental SAH by intraperitoneal injection of sodium pentobarbital (200 mg/kg), and in situ perfusion/fixation was performed. Transcardiac perfusion was performed with 300 mL of normal saline solution followed by 500 mL of ice-cold freshly depolymerized 4% paraformaldehyde in 0.1 mol/L phosphate buffer. Solutions were delivered by a Watson Marlow peristaltic pump at 100 rpm (25 mL/min).

The basilar arteries and the brain stem were harvested en bloc and cryoprotected in 20% sucrose in 0.1 mol/L of

phosphate buffer for 3 days at 4°C. After snap-freezing in dry ice-equilibrated isopentane cooled to -60°C, the specimens were stored at -80°C. The specimens were mounted in tissue freezing compound (Triangle Biomedical Sciences, Durham, NC) and sectioned transversely in 10- $\mu$ m slices with a microtome cryostat (Microm GmbH, Walldorf, Germany) at 200- $\mu$ m intervals beginning at the basilar termination. The tissue slices were mounted on Superfrost Plus slides (Fisher Scientific, Hanover Park, IL) and stained with hematoxylin and eosin followed by coverslip mounting with Permount medium (Fischer Chemicals, Fairlawn, NJ).

Vessel patency was quantified by measuring the basilar artery circumference with the use of a computerized image analysis system (MCID-M5). To correct for vessel deformation and off-transverse sections, the internal circumferences of six different sections of each vessel separated by 200  $\mu$ m were measured and averaged. The luminal cross-sectional area of each vessel was estimated with the use of the calculated radius ( $r$ ) value obtained from the measured circumference ( $r = \text{measured circumference}/2$  ; area of circle =  $r^2$ ).

### Randomized Clinical Study

All patients or their families provided informed written consent. Seventeen consecutive patients admitted with a SAH were enrolled. Patients were randomized to receive the maximum approved dose of simvastatin (80 mg/d) or placebo. Hunt and Hess and Fisher grades were assessed by an independent and blinded neuro-intensivist and neuroradiologist, respectively. Diagnostic cerebral angiography was performed during the first 24 hours after admission. All patients underwent craniotomy and clip ligation within 48 hours of subarachnoid hemorrhage. Nimodipine, phenytoin, and gastrointestinal prophylaxis (H<sub>2</sub>-blockers or proton pump inhibitors) were administered throughout the study period. Decadron was administered before surgery and tapered immediately after surgery. All patients were kept euvoletic during the study period. Serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and creatine-phosphokinase (CPK) were recorded daily to evaluate early signs of hepatitis or myositis secondary to simvastatin administration.

Transaminitis was defined as SGOT and SGPT >180 U/L. CPK increase was defined as CPK >1000. Given the high correlation (94%) of transcranial Doppler (TCD) velocities >200 cm/s with cerebral vasospasm (46), all patients underwent daily TCD evaluation of the middle cerebral, anterior cerebral, and posterior cerebral arteries by a blinded neurologist.

For measurement of serum von Willebrand factor (vWF), S-100 $\beta$ , and TWEAK, blood samples were taken by venipuncture at time of hospital admission and daily thereafter for 14 consecutive days. Collected blood was centrifuged (10,000 g), and the resulting supernatant was immediately frozen at -70°C until analysis was completed. Measurements of vWF, interleukin-8, S-100 $\beta$ , and TWEAK were performed by Biosite Diagnostics (San Diego, CA) using a Genesis Robotic Sample Processor 200/8 (Tecan, Research Triangle Park, NC). All assays were performed in a 10- $\mu$ L reaction volume in 384-well microplates, with the amount of bound antigen detected by means of alkaline phosphatase-conjugated secondary antibodies and AttoPhos substrate (JBL Scientific, San Luis Obispo, CA).

## RESULTS

### Pre-SAH Treatment Studies

In vehicle-treated mice in the large-dose group, SAH caused a 51% reduction in MCA diameter (SAH =  $52 \pm 18 \mu\text{m}$ ;  $n=10$ ; sham =  $105 \pm 6 \mu\text{m}$ ;  $n=9$ ;  $P < 0.001$ ). In simvastatin-treated mice in the same group, SAH caused only a 27% reduction in MCA diameter (SAH =  $74 \pm 22 \mu\text{m}$ ;  $n = 9$ ; sham =  $102 \pm 8 \mu\text{m}$ ;  $n = 9$ ;  $P = 0.003$ ). In mice subjected to SAH, MCA lumen diameter was greater in the simvastatin versus vehicle group ( $P = 0.030$ ). There was no difference between vehicle and simvastatin sham groups ( $P = 0.982$ ). There also was no difference in mean basilar artery diameter between simvastatin-SAH ( $150 \pm 15 \mu\text{m}$ ;  $n = 10$ ) and vehicle-treated SAH mice ( $155 \pm 8 \mu\text{m}$ ;  $n = 9$ ;  $P = 0.527$ ).

In both vehicle- and simvastatin-treated mice, neurologic function worsened 3 days after SAH ( $P < 0.01$ ; Fig. 49.1A). Neurological function was improved in simvastatin-treated versus vehicle-treated SAH mice ( $P < 0.05$ ; Fig. 49.1A). Analysis of all mice revealed that neurologic score correlated with MCA diameter ( $P < 0.01$ ). In eNOS knockout mice, simvastatin (20 mg/kg) had no effect on neurological function 1 to 3 days after SAH compared with vehicle-treated mice (Fig. 49.1A).

In wild-type mice, simvastatin pretreatment increased eNOS expression approximately two- to threefold ( $P < 0.05$ ) versus vehicle in both sham (statin,  $n = 5$ ; vehicle,  $n = 5$ ) and SAH groups (statin,  $n = 5$ ; vehicle,  $n = 5$ ). eNOS expression was unchanged by SAH in vehicle-treated mice. Neurological function was significantly improved 1 to 3 days after SAH ( $P < 0.05$ ) in mice treated with simvastatin (1 mg/kg) versus vehicle (Fig. 49.1B). Three days after SAH, mean motor deficit was reduced by 60% in simvastatin-treated (1 mg/kg) mice. Simvastatin (0.2 mg/kg) was not associated with improved neurological outcome 1 to 3 days after SAH (Fig. 49.1B).

#### Post-SAH Treatment Studies

Body weight did not differ between simvastatin ( $3.45 \pm 0.25 \text{ kg}$ ) and vehicle rabbit groups ( $3.33 \pm 0.46 \text{ kg}$ ). Subarachnoid blood was confirmed within the cisterna magna in all cases. No rabbits died before they were killed for basilar artery harvesting. In vehicle-treated rabbits, SAH ( $n = 5$ ) resulted in  $65 \pm 5\%$  basilar artery lumen patency compared with sham-operated rabbits ( $n = 5$ ;  $P < 0.01$ ). This reduction in basilar artery lumen patency after SAH was attenuated in simvastatin- versus vehicle-treated rabbits ( $83.5 \pm 2\%$  versus  $65 \pm 5\%$ ;  $P < 0.005$ ).

#### Randomized Clinical Study

Seventeen patients with aneurysmal SAH were enrolled in the study. Nine patients were randomized to receive simvastatin, whereas eight received a placebo. Mean  $\pm$  SD age was  $57 \pm 12$  years. Thirteen (76%) patients were women. Median (interquartile range) Hunt and Hess grade was 3 (3–4). Median (interquartile range) Fisher grade was 3 (3). There were no baseline differences in age, Hunt and Hess, or Fisher grade between simvastatin and placebo groups. Two simvastatin patients (22%) developed transaminitis after 1 week of simvastatin treatment. In both cases, transaminitis remained clinically insignificant. CPK increase did not occur in any patients during the study interval.

Mean serum vWF, S-100 $\beta$ , and TWEAK were significantly decreased in patients receiving simvastatin versus placebo Days 3 to 10 post-SAH ( $P < 0.05$ ) (Fig. 49.2). Mean peak S100B level was nearly fourfold lower in simvastatin- versus placebo-treated patients (65 versus 218 ng/mL;  $P < 0.001$ ). Mean peak TWEAK level, an analogue of tumor necrosis factor, was threefold lower in simvastatin- versus placebo-treated patients (0.2 versus 0.6 ng/mL;  $P < 0.001$ ).

Mean peak vWF level was nearly twofold lower in simvastatin- versus placebo-treated patients (15 versus 28 ng/mL;  $P < 0.05$ ).

No patients receiving simvastatin developed TCD evidence of cerebral vasospasm during the study period. However, three patients (38%;  $P = 0.08$ ; Fisher exact test) receiving placebo experienced TCD evidence (velocity  $>200$  cm/s) of cerebral vasospasm.

## DISCUSSION

In this group of experiments, we show that administration of simvastatin before experimental SAH in mice reduced cerebral vasospasm and neurologic deficits 3 days after SAH. Simvastatin pretreatment also produced nearly a threefold increase in cerebrovascular eNOS expression in both control and SAH mice. The efficacy of simvastatin was lost in eNOS-knockout mice, suggesting an eNOS-dependent mechanism of action. In the post-SAH treatment model, simvastatin treatment resulted in a 50% reduction in cerebral vasospasm. In the randomized, placebo-controlled clinical study of 17 patients, concentrations of markers known to be predictive of cerebral vasospasm and neuronal injury, vWF, S-100b, and TWEAK, were significantly reduced in patients receiving simvastatin versus placebo after SAH. Whereas not statistically significant by our criteria, no patient receiving statin therapy developed TCD evidence of cerebral vasospasm compared with three patients in the placebo group. CPK increase or clinically significant transaminitis did not occur in any patient during the study period, suggesting that simvastatin at clinically approved doses (80 mg/d) is safe in the setting SAH. Taken together, these results show a potentially effective and safe role for HMG-CoA reductase inhibitors in the treatment of cerebral vasospasm after SAH.

It is unclear whether the observed efficacy of simvastatin was a result of alterations in vascular smooth muscle tone, changes in brain tissue ischemic threshold by neuroprotective effects, or a combination of both. Other groups have reported ischemic protection and reduction in infarct size with a correspondingly smaller ischemic penumbra with administration of HMG-CoA reductase inhibitors (2, 13). Interestingly, regional blood flow was found to increase after statin treatment in nonpathological conditions (13). Therefore, the neurological benefits observed with simvastatin pretreatment in this study may have resulted from both ischemic protection and improvement in cerebral blood flow.

Numerous other groups have also explored the depletion of eNOS after experimental SAH. Park et al. (40) also observed a reduction in eNOS protein after SAH, but this was seen only 20 minutes after SAH. Similarly, Hino et al. (24) reported an acute 56% reduction in eNOS mRNA but no change in eNOS protein at 7 days after SAH. Kasuya et al. (29) also demonstrated that eNOS protein expression was unchanged 7 days after SAH in a canine basilar artery SAH model. These results suggest that eNOS depletion occurs early and contributes, in part, to the acute phase of cerebral vasospasm but do not explain the vasospasm peak 7 to 10 days after SAH. Prevention of this early eNOS loss with statin treatment, as observed in this and other studies, may explain the marked neurological improvement observed in this study (34). This mechanism is strongly supported by the observed loss of efficacy of statin treatment in eNOS knockout mice.

The safety and potential benefits of HMG CoA reductase inhibitors in the treatment of SAH-induced vasospasm in humans has not been previously explored. Considering the extensive clinical experience in the cardiology literature with these medications (1), their relatively benign side-effect profile (37), and their efficacy in animal studies, it was a logical progression to explore the use of statins in humans with SAH. Our results suggest an important trend toward

TCD reduction in the statin group versus placebo. None of the nine patients in the statin treatment group (average Hunt and Hess = 3) developed TCD evidence of vasospasm versus 38% of the controls.

Additionally, three important serum markers known to be associated with vasospasm and neurological injury (vWF, TWEAK, and S100B) were significantly decreased in the statin group. vWF is a glycoprotein coagulation factor that is released from damaged endothelial cell Weibel-Palade bodies (35, 44). We have previously shown that levels of serum vWF predict timing of cerebral vasospasm (35). TWEAK is a transmembrane protein and a member of the tumor necrosis factor family. These play an important role in the expression of selectins and ICAMs (23), which are crucial for leukocyte diapedesis and extravasation. S100B is a calcium binding protein involved in cytoskeletal rearrangement and has been shown to be increased in association with posthemorrhagic vasospasm and neuronal injury (32). Together, the decreases in these markers with statin therapy provide further evidence of their multifactorial protective effects.

## CONCLUSION

Large-dose simvastatin before SAH attenuated vasospasm and neurological deficits in mice. This effect persisted when simvastatin was given after SAH or at smaller doses approved for human use but was lost in eNOS-knockout mice, suggesting an eNOS-dependent mechanism of action. In humans, simvastatin showed no toxic effects and significantly decreased levels of serum markers of inflammation and endothelial damage that predict vasospasm. Together, these results suggest an important role for statins in the prevention of delayed neurological deficits and cellular injury after SAH.

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FIG. 49.1 A, rotorod latencies were assessed for 3 days after subarachnoid hemorrhage (SAH) to measure neurological deficits in mice (the shorter the Rotorod latency, the more severe the motor deficit). In wild-type mice, neurological deficits were significantly reduced in mice receiving 20 mg/kg of simvastatin versus vehicle after SAH ( $P < 0.01$ ). In eNOS-knockout mice, neurological deficits were similar between simvastatin (20 mg/kg) versus vehicle-treated mice ( $P = 0.746$ ). B, neurological deficits were significantly reduced in mice receiving 1 mg/kg of simvastatin versus

vehicle after SAH ( $P < 0.01$ ). Rotorod latency was similar between simvastatin- (0.2 mg/kg) and vehicle-treated mice ( $P = 0.451$ ). Data presented as mean latency  $\pm$  SEM.

FIG. 49.2 Mean  $\pm$  SEM serum levels of, *A*, von Willebrand factor (vWF), *B*, S-100 $\alpha$  protein, and, *C*, TWEAK Days 1 to 14 after aneurysmal subarachnoid hemorrhage (SAH) in patients receiving simvastatin (80 mg/d;  $n = 9$ ) versus placebo ( $n = 8$ ). Serum vWF, S-100 $\alpha$ , and TWEAK were all significantly lower in patients receiving simvastatin versus placebo 2 to 11 days after SAH ( $P < 0.05$ ). *Black* = simvastatin cohort; *Red* = placebo cohort.