Statins in Traumatic Brain Injury

Elissa F. Wible* and Daniel T. Laskowitz*^{†‡}

*Department of Medicine (Neurology), [†]Department of Anesthesiology, and [‡]Department of Neurobiology, Duke University School of Medicine, Durham, North Carolina 27710

Summary: Traumatic brain injury (TBI) is a common cause of long-term neurological morbidity, with devastating personal and societal consequences. At present, no pharmacological intervention clearly improves outcomes, and therefore a compelling unmet clinical need remains. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or “statins,” offer a potential novel therapeutic strategy for TBI. Statins are well tolerated, easy to administer, and have a long clinical track record in critically ill patients. Their side effects are well defined and easily monitored. Preclinical

studies have shown significant benefit of statins in models of TBI and related disease processes, including cerebral ischemia, intracerebral hemorrhage, and subarachnoid hemorrhage. In fact, multiple mechanisms have been defined by which statins may exert benefit after acute brain injury. Statins are currently positioned to be translated into clinical trials in acute brain injury and have the potential to improve outcomes after TBI. **Key Words:** Statin, HMG CoA reductase inhibitor, closed head injury, neuroinflammation, traumatic brain injury.

INTRODUCTION

Traumatic brain injury (TBI) is one of the most common and financially devastating health problems in our society. There are an estimated 1.5 million cases of TBI annually in the United States, with at least 235,000 resultant hospitalizations and approximately 50,000 fatalities per year.¹ More than 5 million persons in the United States are TBI survivors. Once the acute care period has ended, many TBI patients are left with motor, cognitive, or emotional dysfunction as a result of their injury.² Although several therapies have shown benefit in preclinical models, there has been a notable failure of clinical translation, with a large number of late phase II and III trials failing to confirm benefit in human subjects. Thus, the treatment of TBI remains largely supportive, directed toward management of cerebral edema and intracranial hypertension via temporizing measures, such as administration of osmotic agents, hyperventilation, and ventricular drainage.³ None of these interventions have been definitively demonstrated to improve long-term functional outcome.⁴ The failure of preclinical therapies to translate into clinical benefit may derive from the heterogeneity of TBI pathology, which includes diffuse axonal injury, cerebral contusion, intracerebral hem-

orrhage (ICH), subarachnoid hemorrhage (SAH), and extraparenchymal hemorrhage. These primary insults are exacerbated by a secondary neuroinflammatory cascade of cerebral hypoperfusion and ischemia, oxidative stress, cerebral edema, and intracranial hypertension.

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, also known as “statins,” are an ideal candidate therapy for acute brain injury. Statins influence multiple mechanisms of acute and secondary neuronal injury; they have endothelial and vasoactive properties, as well as anti-oxidant, anti-inflammatory, anti-excitotoxicity, and anti-thrombotic effects. Statin treatment would be practical to implement in TBI because statins have wide availability, Food and Drug Administration approval, a favorable adverse event profile, and a track record of safety in critically ill populations.

CLASSIFICATION OF STATINS

All statins contain an HMG-like component that binds to HMG-CoA reductase.⁵ Other molecular characteristics vary across the class, including potency, lipophilicity, metabolism, and pharmacokinetics. Lovastatin, pravastatin, and simvastatin are obtained from fungi; atorvastatin, rosuvastatin, fluvastatin, and pravastatin are synthetic.⁶ Statin potency refers to the degree of HMG-CoA reductase inhibition. Rosuvastatin is the most potent due to its ability to form multiple

Address correspondence and reprint requests to: Daniel Laskowitz, MD, Duke University Medical Center, Box 2900, Durham, NC 27710. E-mail: danl@neuro.duke.edu.

polar bonds with the HMG-CoA reductase enzyme. Atorvastatin is the next most potent, followed by simvastatin, fluvastatin, and pravastatin.⁵ Whether the statin potency is important in neuroprotection after TBI is unknown, as many of the proposed mechanisms are not related to HMG-CoA reductase inhibition. Pravastatin and rosuvastatin are hydrophilic due to polar groups, whereas lovastatin, atorvastatin, fluvastatin, and simvastatin are lipophilic.^{5,6} The lipophilic drugs tend to diffuse more readily across cell membranes, thereby making these drugs more readily absorbed into tissues to affect intracellular processes or cause toxicity. Statins are variably metabolized by the cytochrome P₄₅₀ (CYP450) system; statins using these pathways are more likely to cause myopathy because other drugs also using the same pathway may increase statin plasma levels. The lipophilic statins more often use the CYP450 system and therefore have a higher incidence of myopathy.⁶ Simplistically, bioavailability is the amount of drug available to nonhepatic cells. Cerivastatin is the most bioavailable at 60% and was withdrawn from the market due to high rates of myopathy. The rest of the group is between 14 and 24% bioavailable, with the exceptions of lovastatin and simvastatin, with less than 5% bioavailability.^{5,6} Rosuvastatin and atorvastatin have a long half-life (20 and 14 hours, respectively) compared with the remaining statins (<3 hours).^{5,6}

EFFICACY OF STATINS IN PRECLINICAL MODELS OF ACUTE BRAIN INJURY

Statins are potent inhibitors of cholesterol biosynthesis⁷ via HMG-CoA reductase inhibition. Statins are widely used to decrease low-density lipoprotein (LDL) levels and lower the risk of cardiovascular events. As clinical experience with statins has increased, evidence suggests that the cardiovascular benefit may not solely be related to cholesterol lowering, but also to systemic and vascular anti-inflammatory effects. There is growing evidence that statins have additional properties that are neuroprotective, also independent of serum cholesterol effects.⁸ The therapeutic effects of statins in brain injury may be divided according to mechanism from most acute to more chronic: acute lesional effects, anti-inflammatory and anti-excitotoxic effects, vascular and endothelial effects, anti-apoptotic effects, and effects on neurogenesis and angiogenesis (FIG. 1, Table 1).

Acute lesional effects

There is some weak evidence from preclinical studies implying that statins may modulate the initial parenchymal damage in TBI. For example, cortical contusional volume was decreased when rats were treated with lovastatin prior to experimental TBI,⁹ although this result was not replicated with simvastatin or atorvastatin.¹⁰ When animals were treated with statins post-injury, no

FIG. 1. Statin effects in traumatic brain injury (TBI). Bax/Bcl-2 = Bcl-2 associated X protein; BBB = blood brain barrier; BDNF = brain-derived neurotrophic factor; eNOS = endothelial isoform of nitric oxide synthase; PKB = protein kinase B; PI3K = phosphoinositide-3-kinase; VEGF = vascular endothelial growth factor; VEGFR2 = vascular endothelial growth factor receptor 2; vWF = von Willebrand Factor.

Table 1. *Animal Models Testing Statins in Traumatic Brain Injury*

Reference (Date)	Type of Injury	Animal	Histologic Outcomes	Functional Outcomes
Lu et al. ¹¹ (2004)	CCI	Rat	Increased perilesional and hippocampal neuron survival, increased neuronal synapses, increased angiogenesis	Improved motor function at days 4–14
Lu et al. ¹² (2004)	CCI	Rat	Increased rate of hematoma resorption	NT
Lu et al. ⁴⁰ (2004)	CCI	Rat	Decreased vessel thrombosis	NT
Lu et al. ⁴¹ (2004)	CCI	Rat	Decreased intravascular thrombosis	Improved spatial memory
Qu et al. ⁴³ (2005)	CCI	Female rat	Increased hippocampal and perilesional neuron survival; increased neuronal process survival in hippocampus only; increased vessel density	Improved spatial memory at day 15, no change in sensorimotor function
Lu et al. ⁴⁴ (2007)	CCI	Rat	Increased hippocampal neuron survival; increased neurogenesis; increased angiogenesis	Improved spatial learning at days 31–35
Wang et al. ¹⁰ (2007)	CHI	Mouse	Decreased glial activation; decreased TNF α and IL-6; no change in eNOS	Improved motor function at day 5, improved spatial learning at day 24
Chen et al. ¹⁹ (2008)	CCI	Rat	Decreased cerebral edema	Improved motor function at day 1, but not at days 3–7
Mahmood et al. ⁵⁹ (2008)	CCI	Rat	Increased cellular proliferation	Improved motor function at days 7–90
Wu et al. ⁴⁵ (2008)	CCI	Rat	Decreased apoptosis	Improved motor function at days 7–35
Wu et al. ⁴⁶ (2008)	CCI	Rat	Increased neurogenesis, increased BDNF and VEGF	Improved spatial learning at days 34–35
Chen et al. ¹⁶ (2009)	CCI	Rat	Decreased cerebral edema, decreased BBB permeability, decreased apoptosis	Improved motor function at day 1
Turkoglu et al. ¹⁸ (2009)	CCI	Rat	Decreased cerebral edema, lipid peroxidation, and degeneration of myelinated axons	NT

BBB = blood-brain barrier; BDNF = brain-derived neurotrophic factor; CCI = controlled cortical impact; CHI = closed head injury; eNOS = endothelial isoform of nitric oxide synthase; IL-6 = interleukin-6; mNSS = modified neurological severity scores; NT = not tested; TNF α = tumor necrosis factor- α ; VEGF = vascular endothelial growth factor.

difference was seen in the acute lesional volume.¹¹ In another animal TBI model, atorvastatin increased the rate of resorption of intraparenchymal and intraventricular hematomas, although no difference in hematoma volume was seen initially or at 15 days post-injury.¹² Because the initial mechanical insult accounts for only a small part of the ultimate injury in TBI, and statins, at best, only modestly change the initial contusions and hematomas, it seems unlikely that statins change the initial parenchymal damage to any clinically significant degree.

Effects on inflammation and excitotoxicity

The neuroinflammatory response after TBI causes secondary neuronal cell death subacutely via excitotoxic injury, lipid peroxidation, blood-brain barrier (BBB) breakdown, and cerebral edema. Edema and intracranial pressures (ICP) usually peak 72 to 96 hours after injury. Ventricular drainage of CSF, osmotic agents, hyperventilation-induced cerebral vasoconstriction, and induced hypothermia are effective in transiently reducing ICP.¹³ However, none of these interventions has been defini-

tively demonstrated improved survival or functional outcome.⁴ Similarly, no immunomodulatory interventions have shown benefit in TBI, and some strategies (such as the administration of glucocorticoids) are associated with worse outcomes.¹⁴ Thus, a better understanding of how to selectively dampen the destructive effects of neuroinflammation would hold great promise in developing new therapeutic strategies.

Statins have been demonstrated to exert an anti-inflammatory response in the injured CNS, in part by decreasing the formation of isoprenoids, which are intermediate structures in cholesterol metabolism and play an important role in mediating inflammatory responses. In fact, a number of preclinical brain injury models have demonstrated that statin administration is associated with reduced inflammatory mediators, glial activation, cerebral edema, and increased BBB integrity.

Preclinical models of TBI have demonstrated a post-traumatic upregulation of inflammatory mediators. In particular, tumor necrosis factor- α (TNF- α),^{9,15} interleukin-6 (IL)-6,¹⁵ and IL-1 β ⁹ are increased and associated with the loss of BBB integrity, likely contributing to cerebral edema. Recently, the toll-like receptor (TLR) 4 pathway has been proposed as the mechanism by which these mediators cause inflammation after TBI.¹⁶ TLR4 is activated by molecular products of injury, including heat-shock proteins,¹⁷ intracellular molecules from ruptured cells, and products of cell degradation or proteolysis. TLR agonism is associated with nuclear translocation of nuclear factor- κ B, a transcription factor that is associated with the upregulation of inflammatory mediators, including IL-1 β , TNF- α , IL-6, and intracellular adhesion molecule-1 (ICAM-1).¹⁶ In a rat TBI model, simvastatin, given after cortical contusion, decreased both the mRNA and protein expressions of TLR4 and nuclear factor- κ B.¹⁶ In both pre-injury and post-injury treatment models, animal studies have demonstrated that statin treatment decreases IL-1 β ,^{9,16} TNF- α ,^{9,10,16} IL-6,^{10,16} and ICAM-1¹⁶ levels in the acute or subacute period after traumatic injury.

A reduction in neuroinflammatory mediators may also be associated with stabilization of the BBB after trauma, an effect that is consistent with a reduction in cerebral edema observed in several animal TBI models.^{16,18,19} In cultures of human BBB endothelia, lovastatin and simvastatin were shown to decrease the translocation of both albumin and sucrose, but not normal leukocytes.²⁰ Microglia (i.e., the resident immune cells of the brain) are implicated in the secretion of glutamate, reactive oxygen species, and other inflammatory mediators, which may exacerbate cerebral edema and secondary neuronal injury. Microglial markers increase after experimental TBI, peak at 24 hours, and continue for 7 days. The administration of atorvastatin significantly decreased this response at all time points measured.¹⁰ In addition to

reducing oxidative stress via downregulation of microglial activity, statins decreased the production of other harmful oxygen free radicals, such as superoxide, when baseline inflammation was present.²¹

Various statins have been shown in large clinical trials to decrease systemic markers of inflammation, such as high sensitivity C-reactive protein.^{22–24} However, clinical data in TBI remains preliminary. In the only prospective study of statins in TBI patients, 8 were treated with rosuvastatin and 13 were given a placebo. No difference was found between the groups in the median IL-1 β or TNF- α levels; in fact, contrary to animal models, median IL-6 levels were increased in the statin-treated group.²⁵ However, it is worth noting that rosuvastatin has limited CNS penetration, and thus it is difficult to draw definitive conclusions for the effect of statins on CNS inflammation based on of this small pilot study.

Excitotoxicity may also contribute to secondary early neuronal loss after TBI. Excitatory neurotransmitters, such as glutamate, are released from damaged cells. Glutamate binding to N-methyl-D-aspartate (NMDA) receptors leads to sodium ion influx with further neuronal depolarization. Calcium influx also increases, activating intracellular calcium-dependent catalytic enzymes and leading to neuronal autophagy.²⁶ Statins have been associated with a reduction of glutamate excitotoxicity, either via changes in the cell cholesterol metabolism,^{27,28} activation of TNF receptor 2 signaling,²⁹ or other as yet unknown mechanisms.²⁶

Although many proposed statin effects are cholesterol-independent, statins may exert a neuroprotective effect through decreased cholesterol production. In neuronal cell culture, simvastatin decreased intracellular cholesterol levels and decreased NMDA-receptor associated cell death, but did not change NMDA receptor levels.²⁸ Because approximately 60% of the NMDA receptors are associated with cholesterol-rich lipid rafts in the cell membrane, one possible mechanism of statin-induced neuroprotection is a decreased association of the NMDA receptors with these lipid rafts. This might be due to an internalization of receptors, deeming them nonfunctional, or a change in protein configuration and functionality when the receptors are not associated with the lipid rafts.²⁸

Vascular and endothelial effects

Statins positively affect the vasculature by improving endothelial function and reducing microthrombosis. This is a potentially important mechanism, as cerebral hypoperfusion and ischemia can exacerbate neuronal injury after TBI and impaired vascular responses have been described in both preclinical and clinical settings.³⁰ In laboratory studies, statins are associated with a reduction in post-traumatic hypoperfusion and rebound hyperemia.¹⁰ Cerebral autoregulation is impaired after TBI,

with the pial arterioles unable to respond to changes in PCO_2 and PO_2 .³¹ The lack of normal vasomotor reactivity renders the already compromised brain exquisitely sensitive to modest hypoxemia or relative hypotension that would otherwise be well tolerated. In fact, episodes of hypotension are common in the head-injured population and are significantly associated with poor outcomes.^{32,33}

Vascular endothelium regulates smooth muscle tone via nitric oxide (NO) pathways. The endothelial isoform of nitric oxide synthase (eNOS) is constitutively expressed in the cerebrovascular endothelium and mediates vasodilation. Statins selectively upregulate eNOS, independent of changes in serum cholesterol.^{34,35} Statin treatment has been shown to increase eNOS mRNA, protein, and enzymatic activity up to three-fold, resulting in an increase in cerebral blood flow.^{36–39} However, the data is mixed, as decreased eNOS RNA levels were found in one murine model of TBI, and eNOS RNA levels were unchanged by statin treatment.¹⁰ Therefore, the exact role of eNOS in TBI is unclear, although it remains possible that statins could affect the eNOS system downstream from transcription.

Statins also reduce intravascular thrombosis in preclinical models.⁴⁰ In a rat TBI model, thrombosed vessels were seen post-injury bilaterally throughout the brain, most notably in the contused cortex, peri-contusional boundary zone, and ipsilateral hippocampus. Intravascular thrombosis was present as soon as 1 hour, peaked at 4 to 72 hours, and returned to controlled levels within 15 days. Specifically, thrombosis increased until day 3 in the peri-contusional areas and ipsilateral hippocampus, referred to as delayed thrombosis.⁴⁰ Administration of atorvastatin within 24 hours of injury decreased the level of delayed thrombosis significantly at days 3 and 8 post-TBI.^{40,41} This statin-associated decrease in intravascular thrombosis correlated with a reduction in necrotic brain tissue,⁴¹ suggesting that the thromboses led to a critical reduction in tissue perfusion. Pathologically, thromboses include platelets, fibrin, and von Willebrand factor. An increase in systemic platelet activity and von Willebrand factor levels mirrored the time pattern of thrombosis, also peaking at days 1 to 3, and being significantly decreased by atorvastatin treatment.⁴⁰ These preclinical studies are consistent with clinical evidence suggesting that statins decrease thrombotic markers in other forms of acute brain injury. For example, a significant reduction in circulating von Willebrand factor was seen in patients treated with simvastatin after aneurysmal SAH, which could potentially reduce microvascular dysfunction.⁴² Other potential mechanisms of enhanced perfusion associated with statins include the downregulation of adhesion molecules and stabilization of endothelial function.

Effects on apoptosis

A reduction in programmed cell death is another mechanism by which statins may improve outcomes. TBI causes both a loss of neurons and a decrease in the normal neuronal architecture in the peri-contusional areas and ipsilateral hippocampus. Various animal models have shown improvement of neuron survival in both areas with statin administration.^{10,11,43,44} Neuronal loss may continue up to 35 days post-TBI, and statin treatment decreases the degree and rate of hippocampal neuron loss.⁴⁴ Statins suppress the activation of caspase-3, a key protease in the apoptotic pathway, as early as 1 day after statin treatment. Apoptotic cell death was subsequently decreased by statin treatment in the ipsilateral hippocampus and peri-contusional cortex.⁴⁵ In addition, the ratio of Bax/Bcl-2 is significantly reduced in simvastatin-treated animals, favoring an anti-apoptotic environment; this reduction was associated with an improvement in delayed cognitive function.⁴⁴

Effects on neurogenesis and synaptogenesis

In addition to protecting existing neurons from apoptosis after TBI, there is evidence that statin administration promotes the growth and differentiation of new neurons. Animal TBI models demonstrate new cells with markers of neuronal differentiation, suggesting that TBI itself induces neurogenesis.^{11,44} Statin treatment increases cells with markers of proliferation found in the hippocampus, and more of these new cells differentiate into neurons.^{44,46} This effect persists as long as 35 days after brain injury.⁴⁴ The increase in neurogenesis may be related to an upregulation of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF). Statins increase BDNF in peri-ischemic zone endothelial cells in animal models of stroke. Neuronal cell migration in cell culture is enhanced by statins and suppressed by anti-BDNF antibodies, suggesting BDNF-related mechanism to neuronal migration.⁴⁷ Consistent with the data from the stroke model, BDNF was increased in the hippocampi of statin-treated animals at day 7 after TBI.⁴⁶ Additionally, although VEGF is named for its promotion of angiogenesis, it also increases neurogenesis and axonal sprouting.⁴⁷

The phosphoinositide-3-kinase/Akt pathway is one possible mechanism by which neurotrophic factors, such as BDNF, may be upregulated. The phosphoinositide-3-kinase/Akt pathway is important in neuronal cell growth, cell survival, neuronal differentiation, and protein synthesis.^{45,48} Preclinical studies have demonstrated that statins increase phosphorylation of this pathway.⁴⁶ In an animal TBI model, simvastatin treatment improved histological and functional outcomes, increased Akt activation at days 1 through 7, and increased downstream

targets of the Akt pathway in the hippocampi and cortices.⁴⁶ In addition to increasing neurotrophic factors, Akt activation shifts cells away from apoptosis. Phosphorylation of Akt leads to the inactivation of pro-apoptotic proteins, such as Bcl-2⁴⁹ and forkhead transcription factor (FOXO1), and activation of anti-apoptotic proteins inhibitory- κ B (I κ B) and eNOS.⁴⁵ Therefore, statin induction of Akt phosphorylation may affect multiple systems to improve functional outcomes after TBI. Other neurotrophic mechanisms promoted by statins, which have animal or cell culture support, include the modulation of neuromigratory proteins (doublecortin and tubulin isotype II).⁴⁷

This upregulation of neurotrophic factors and neurogenesis has also been associated with increased synaptogenesis. Synaptophysin is associated with the presynaptic terminal and is decreased in the peri-contusional cortex. Statin treatment increases synaptophysin staining in the peri-contusional cortex and ipsilateral hippocampus, reflecting either a protective effect from secondary injury on the synapse or an increase in synaptogenesis.¹¹ Animal ischemic stroke models have indicated similar results, showing that low-dose statins increase synaptophysin and activate cortical neuronal phosphoinositide-3-kinase/Akt and downstream Erk pathways.⁵⁰ Increased synaptogenesis after statin use may be mediated by phosphorylated Akt, which is important for synaptic plasticity and memory consolidation.⁴⁹

Effects on angiogenesis

Statins have been shown to promote angiogenesis in both TBI and ischemic stroke models.^{47,50} Overall, the upregulation of VEGF and an increase in NO/eNOS system may be the mechanisms by which statins increase angiogenesis. In stroke models, statins increase angiogenesis in the ipsilateral hemisphere^{47,50} and significantly increase VEGF^{47,50,51} and VEGF receptor 2 (VEGFR2)⁴⁷ within the ischemic penumbra. Antagonism of either the VEGF system^{47,50} or the NO system⁵⁰ in cell culture decreases this statin-induced angiogenesis. The increase in blood vessel growth appears to be independent of cholesterol-lowering, as it occurs even in normocholesterolemic animals, an effect presumably mediated via the activation of Akt and subsequently eNOS.⁵² The NO then leads to angiogenesis by enhancing endothelial cell proliferation and migration.⁵³ TBI models have similarly demonstrated increased angiogenesis and VEGF levels with statins.⁴⁶ Contused murine cortex has decreased density, diameter and length of blood vessels, but statins partially ameliorate these effects.^{41,43} Statins increase capillary density and newly-formed vessels in the pericontusional cortex and hippocampus.^{11,44} However, care must be taken in determining statin dosage, as there appears to be a biphasic effect of statins on angiogenesis in both cell culture and animal ischemic stroke

models; low-dose statins typically increase angiogenesis, whereas high-dose statins may inhibit angiogenesis.^{50,51}

PRECLINICAL MODELS OF ACUTE BRAIN INJURY

Because TBI is inherently heterogeneous, any potential treatment should be shown to be efficacious across multiple different preclinical models of acute brain injury prior to human trials. Traditionally, head injury models are performed in rodents; a craniotomy allows for a reproducible injury, such as fluid-percussion or controlled cortical impact, to be directly applied to the brain parenchyma.⁵⁴ This type of model creates a reproducible and well-defined area of tissue injury. However, these injuries may not be clinically relevant, as human TBIs often occur through an initially intact skull with energy transfer from the skull to the intracranial contents. In addition, rapid acceleration–deceleration forces, such as those that occur during a motor vehicle collision, combine with torsional forces to produce a shearing of the long white matter tracks or diffuse axonal injury. Other tissue injuries include cortical contusion and hemorrhage, subarachnoid hemorrhage, intraventricular hemorrhage, and subdural and epidural hematoma. To address the limitations of open craniotomy models, weight-drop models were constructed to more closely mimic an impact against the closed skull. A recently developed technique uses a calibrated, stereotactically guided, pneumatic impact to the closed skull.^{10,55–57} Indeed, more clinically relevant injuries are produced, along with the resultant short-term neurologic and longer-term cognitive deficits.⁵⁸

Statins have demonstrated benefit in a variety of animal TBI models. The improvement in biochemical and histological markers seen with statins after experimental TBI are supported by better functional outcomes. Improved performance on neurological severity score, Rotarod latency, corner turn, and Morris water maze testing have all been demonstrated after statin treatment.^{9–11,41,45,46,59} The beneficial effect of statins appear to be a class effect, with the exception of a study reported by Lu et al.,⁴⁴ who found a statistically significant improvement with simvastatin but not atorvastatin. Gender effects have not fully been studied, as most models to date have been performed in male animals. In a study of female rats, statin-treated animals performed better than the control group in tests of spatial memory at 2 weeks post-injury, but had no improvement at any time point in sensorimotor function.⁴³ Previously, the same authors had shown improvement in both spatial memory and sensorimotor function in a parallel study of male rats.¹¹

One advantage to preclinical modeling is that different features of TBI pathology may be recreated. As previously described, TBI pathology is often heterogeneous.

Table 2. *Prospective Randomized Clinical Trials of Statins after Acute Brain Injury*

Reference (Date)	Disease Process	Total (n)	Trial Design	Dosing Paradigm	Clinical Outcomes
Lynch et al. ⁴² (2005)	SAH	39	Prospective, randomized, placebo-controlled	Simvastatin, 80 mg daily for 14 days	Decreased vasospasm, decreased DIDs
Tseng et al. ⁷⁵ (2005) and Tseng et al. ⁹⁴ (2007)	SAH	80	Prospective, randomized, placebo-controlled	Pravastatin, 40 mg daily for 14 days	Decreased vasospasm, decreased DIDs, decreased mortality. Improved favorable outcome (mRS 1 or 2) at 6 months.
Blanco et al. ⁹¹ (2007)	Ischemic stroke	89	Prospective, randomized	For patients on chronic statin therapy: atorvastatin, 20 mg daily for 3 days vs statin withdrawal for 3 days, and then atorvastatin, 20 mg daily for at least 3 months	In the statin-withdrawal group: increased mortality and increased early neurologic deterioration
Chou et al. ⁷⁶ (2008)	SAH	39	Prospective, randomized, placebo-controlled	Simvastatin, 80 mg daily for as many as 21 days	No difference in mortality, angiographic vasospasm, or DIDs
Montaner et al. ⁹⁵ (2008)	Ischemic stroke	60	Prospective, randomized, placebo-controlled	Simvastatin, 40 mg daily for 7 days, then 20 mg daily until day 90	No difference in mRS or NIHSS at 3 months
Tapia-Perez et al. ²⁵ (2008)	TBI	21	Prospective, randomized, placebo-controlled	Rosuvastatin, 20 mg daily for 10 days	Improved amnesia and orientation. No difference in DRS at 3 months
Vergouwen et al. ⁷⁷ (2009)	SAH	32	Prospective, randomized, placebo-controlled	Simvastatin, 80 mg daily for 14 days	No difference in vasospasm, DIDs, or GOS at 3 or 6 months

DIDs = delayed ischemic deficits; DRS = disability rating score; GOS = Glasgow outcome score; mRS = modified Rankin scale; NIHSS = National Institutes of Health stroke scale; SAH = subarachnoid hemorrhage; TBI = traumatic brain injury.

Preclinical data supports the benefit of statins in many of these disease processes. The most compelling preclinical data is in experimental SAH, where statins have been demonstrated to reduce vasospasm and improve outcomes after SAH in the mouse,^{39,60} rat,^{61,62} rabbit,^{39,63} and dog.⁶⁴ Similarly, statin treatment has been shown to improve outcomes in murine models of intracranial hemorrhage^{65,66} and acute ischemic stroke.^{67–69}

CLINICAL EXPERIENCE WITH STATINS FOR THE TREATMENT OF ACUTE BRAIN INJURY

Preclinical models clearly show a benefit of statins in TBI. However, clinical data remains sparse (Table 2). There are no large prospective studies of statins in moderate to severe TBI. Recently, a small prospective, ran-

domized, double-blind trial compared 8 patients who had moderate TBIs and received rosuvastatin (20 mg daily for 10 days) starting within 24 hours of injury with 13 patients who had comparable injuries, but were given a placebo. Rosuvastatin treatment was associated with a decreased duration of amnesia, although there were no differences in 3-month outcomes in this small pilot study. Of importance, the authors note that the rosuvastatin group had a somewhat worse degree of TBI at baseline than the placebo group.²⁵

After preclinical data suggesting neuroprotection, statins have also been considered in other forms of acute brain injury commonly associated with TBI. Clinical data on statins in ICH is mixed. A small retrospective study of ICH found that pre-stroke statin use was associated with a reduction in mortality⁷⁰ and perihematomal

edema.⁷¹ Similarly, from the prospective Israeli stroke registry, patients with ICH on chronic statin therapy had less neurologic deficit on admission, an increased likelihood of a good neurologic outcome, and decreased mortality, despite having more comorbidities and a higher rate of warfarin therapy.⁷² However, a larger retrospective study found no difference in ICH volume or outcomes associated with previous statin use.⁷³ In the setting of acute ischemic stroke, preclinical data suggests that the acute administration of statins may also be associated with neuroprotection, and the safety and feasibility of this approach is currently being evaluated in a pilot clinical study.⁷⁴

At present, the best prospective data in critically ill patients with acute brain injury is in aneurysmal SAH, in which two small, prospective clinical studies demonstrated a benefit with statins. In 2005, Lynch et al.⁴² found that simvastatin (80 mg daily for 14 days) reduced the incidence of vasospasm and biomarker surrogates of endothelial dysfunction and CNS inflammation. These results were concordant with the study by Tseng et al.,⁷⁵ who demonstrated that pravastatin (40 mg daily for 14 days) improved surrogate measures of vasospasm and functional outcomes. Two other small prospective studies also demonstrated safety and feasibility of statin use in SAH, but did not show statistically significant improvement in vasospasm, delayed ischemic deficit, or functional outcomes.^{76,77} It is worth noting that practice patterns for SAH treatment in many institutions had already changed to include statins after the first two studies were reported, despite mixed evidence in subsequent retrospective^{78,79} and prospective cohort studies.^{80,81} Hopefully the role of statins in acute SAH will be clarified by the prospective, multicenter SimvaSTatin in Aneurysmal Subarachnoid Hemorrhage (STASH) trial.^{82,83}

FUTURE DIRECTIONS

Combination therapy

Despite the heterogeneity of TBI and its secondary responses, most therapeutic trials to date have tested an individual therapy with one focused mode of action. In 2008, the National Institute of Neurological Disorders and Stroke (NINDS), with support from the National Institute of Child Health and Development, the National Institute of Heart, Lung, and Blood, and the Department of Veterans Affairs convened a workshop to discuss the opportunities and challenges of testing combination therapies for TBI.⁸⁴ Given their favorable adverse event profile and pleiotrophic effects, statins are an excellent candidate for combination therapy.

One example of combination therapy is the concomitant use of statins and fibrates, which are peroxisome proliferator-activated receptor α agonists. Peroxisome proliferator-activated receptor α is a nuclear receptor that

regulates glucose metabolism, lipoproteins, and lipids, and has multiple effects on inflammatory and oxidative mediators. The combination of statins with fibrates synergistically increases peroxisome proliferator-activated receptor α activity and inhibits nuclear factor- κ B. Fenofibrate alone decreases cerebral edema, inflammatory markers, and the volume of cerebral injury, while improving the neurologic functional score in rats after experimental TBI.⁸⁵ Because statins and fibrates can have synergistic effects, the combination was tried. Rats given both fenofibrate and simvastatin at 1 and 6 hours post-injury had improved neurologic function at 2, 3, and 7 days compared to the controls. Monotherapy with either agent initially improved neurologic function, but did not improve outcomes at 3 or 7 days. Finally, if treatment was delayed by 3 and 8 hours post-injury, benefit in neurologic function was only seen with the fibrate-statin combination.¹⁹

Simvastatin and marrow stem cells have also been shown to improve the functional outcome of rats up to 90 days after experimental TBI, both when used as a monotherapy and synergistically when given sequentially. The synergistic effect may be mediated by increased delivery of marrow stem cells to the peri-contusional area by statin-induced angiogenesis, and subsequent activation and survival of marrow stem cells secondary to trophic factors released from the new blood vessels.⁵⁹

Clinical trial designs for statins in TBI

There are many practical and theoretical advantages to using statins for acute brain injury. Statins are well tolerated, easy to administer, and have a long clinical track record of safety in critically ill patients. Their side effects are well defined and easily monitored. Before designing a clinical trial to definitively answer whether statins have a role in the management of TBI, several variables must be clarified, including which statin should be used, the dosage, the timing of initial therapy, and the duration of treatment.

The choice of a particular statin for use in a TBI study is clearly an outstanding question. The palliative effect of statins most likely represents a class effect. Preclinical studies of TBI have used mostly atorvastatin^{10–12,41,43,44,61} and simvastatin,^{10,16,19,45,46,59,64,86} which are relatively lipophilic, and therefore have better BBB penetration.⁸⁷ Hydrophilic statins have minimal penetration across the BBB.⁸⁷ However, neuroprotection has been demonstrated even with hydrophilic statins, such as rosuvastatin⁶⁷ and pravastatin,⁷⁵ presumably due to vascular and anti-inflammatory effects, although breach of the BBB is common after acute brain injury. In fact, the pilot trials evaluating statins in SAH used both lipophilic and hydrophilic statins (simvastatin⁴² and pravastatin,⁷⁵ respectively), with a similar decrease in cerebral vasospasm. It is not known whether potency or

the ability of a particular statin to lower cholesterol is important in the setting of neuroprotection. Indeed, statins across the potency spectrum have been shown to be effective in animal models of stroke and TBI. Pharmacokinetic properties may also be an important consideration in designing a clinical trial, which would favor statins with a longer half life, such as atorvastatin and rosuvastatin. Another consideration is the metabolism of simvastatin and atorvastatin by the CYP3A4 enzymatic pathway. Rosuvastatin, fluvastatin, and pravastatin do not use this pathway and are therefore subject to fewer interactions with antifungals, macrolides, calcium channel blockers, and other drugs.⁵ This may be particularly relevant in critical care, as polypharmacy is common.

Potential adverse effects of statins (most commonly myositis and transaminitis) should be closely monitored during clinical translation. Fortunately, these effects tend to be self-limited and are easily monitored. Moreover, statins have been demonstrated to be safe in a critically ill patient population with acute brain injury.^{42,75,76} Patients should also be closely evaluated for other potential adverse effects of statins in the setting of acute brain injury. For example, it was recently discovered that chronic simvastatin treatment increased demyelination and decreased subsequent remyelination in a murine multiple sclerosis model, and this was unexpected as it was not observed during acute treatment.⁸⁸ Questions also remain regarding long-term therapy in patients prone to ICH, as statin therapy and/or low LDL may increase the risk of primary ICH⁸⁹ or hemorrhagic transformation of ischemic tissue.⁹⁰

The timing and duration of statin administration also appears to be important. Many preclinical studies administered statins within hours to 1 day after TBI, and so the time window for initial drug administration is unclear. Randomization into two dosing arms, one hyperacute and one acute, would be one way to delineate the optimal timing of the first dose. Although there is evidence of acute effects of statins, there are also likely benefits from subacute and chronic mechanisms, and this would favor a longer time window for initial medication administration. The optimal duration of statin therapy is also unknown. Pilot trials of statins in SAH gave 14 days of therapy,^{42,75} as the majority of cerebral vasospasm occurs within this time. Duration of therapy in preclinical trials is variable. Although most studies administer only 3 to 14 days of statin therapy, improvement in motor and cognitive function after this treatment has been demonstrated up to 35 days after injury.^{44–46} Therefore, measurements of long-term outcomes are important, even after a short drug course. Finally, clinical trials in both stroke and SAH suggest the possibility of a rebound effect and worsening outcomes when statins are withdrawn.^{75,91,92} Future studies should carefully consider the potential adverse effects of randomizing patients on

chronic statin therapy to a control group or the premature termination of statin treatment.

Thus, despite the clear promise of HMG CoA reductase inhibitors for TBI, a number of unanswered questions remain prior to clinical trial design. Some of these challenges are inherent in TBI trials, such as the difficulty in adequately powering a randomized clinical trial and choosing sensitive functional endpoints.⁹³ It would be advantageous to include surrogate endpoints in early trials to establish benefit on specific mechanisms of action. Short-term biochemical and clinical surrogates for TBI could include markers of cerebral edema, intracranial pressure, endothelial function, and/or neuroinflammation. Long-term motor and cognitive endpoints should be included to establish effects on chronic recovery of function. Ultimately, the definitive clinical trials will need to define the effects of statins on clinically relevant and sensitive functional outcomes.⁹³

CONCLUSIONS

The use of statins remains a novel therapeutic strategy for TBI. There is robust preclinical data demonstrating the efficacy of statins in acute brain injury models that recapitulate the heterogeneous pathology of clinical TBI. Animal studies have defined mechanisms by which statins may improve outcomes after TBI and should guide statin choice and dosing paradigm for clinical translation. Future prospective clinical trials should incorporate acute surrogate biochemical or clinical endpoints as well as define subacute clinical functional outcomes. Thus, although statins represent a promising new therapy in an area with compelling unmet clinical need, additional preclinical and prospective clinical studies are necessary before this potential can finally be realized.

REFERENCES

1. Rutland-Brown W, Langlois JA, Thomas KE, Xi YL. Incidence of traumatic brain injury in the United States, 2003. *J Head Trauma Rehabil* 2006;21:544–548.
2. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil* 2006;21:375–378.
3. Bulger EM, Nathens AB, Rivara FP, Moore M, MacKenzie EJ, Jurkovich GJ. Management of severe head injury: institutional variations in care and effect on outcome. *Crit Care Med* 2002;30:1870–1876.
4. Biros MH, Heegaard W. Prehospital and resuscitative care of the head-injured patient. *Curr Opin Crit Care* 2001;7:444–449.
5. McKenney JM. Pharmacologic characteristics of statins. *Clin Cardiol* 2003;26:III32–III38.
6. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol* 2005;19:117–125.
7. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990;343:425–430.
8. Cucchiara B, Kasner SE. Use of statins in CNS disorders. *J Neurol Sci* 2001;187:81–89.
9. Chen SF, Hung TH, Chen CC, et al. Lovastatin improves histological and functional outcomes and reduces inflammation after experimental traumatic brain injury. *Life Sci* 2007;81:288–298.

10. Wang H, Lynch JR, Song P, et al. Simvastatin and atorvastatin improve behavioral outcome, reduce hippocampal degeneration, and improve cerebral blood flow after experimental traumatic brain injury. *Exp Neurol* 2007;206:59–69.
11. Lu D, Goussev A, Chen J, et al. Atorvastatin reduces neurological deficit and increases synaptogenesis, angiogenesis, and neuronal survival in rats subjected to traumatic brain injury. *J Neurotrauma* 2004;21:21–32.
12. Lu D, Mahmood A, Qu C, Goussev A, Lu M, Chopp M. Atorvastatin reduction of intracranial hematoma volume in rats subjected to controlled cortical impact. *J Neurosurg* 2004;101:822–825.
13. Silvestri S, Aronson S. Severe head injury: prehospital and emergency department management. *Mt Sinai J Med* 1997;64:329–338.
14. Alderson P, Roberts I. Corticosteroids for acute traumatic brain injury. *Cochrane Database Syst Rev* 2005;CD000196.
15. Ramilo O, Saez-Llorens X, Mertsola J, et al. Tumor necrosis factor alpha/cachectin and interleukin 1 beta initiate meningeal inflammation. *J Exp Med* 1990;172:497–507.
16. Chen G, Zhang S, Shi J, Ai J, Qi M, Hang C. Simvastatin reduces secondary brain injury caused by cortical contusion in rats: possible involvement of TLR4/NF-kappaB pathway. *Exp Neurol* 2009;216:398–406.
17. Tsan MF, Gao B. Endogenous ligands of Toll-like receptors. *J Leukoc Biol* 2004;76:514–519.
18. Turkoglu OF, Eroglu H, Okutan O, et al. Atorvastatin efficiency after traumatic brain injury in rats. *Surg Neurol* 2009;72:146–152.
19. Chen XR, Besson VC, Beziaud T, Plotkine M, Marchand-Leroux C. Combination therapy with fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, and simvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, on experimental traumatic brain injury. *J Pharmacol Exp Ther* 2008;326:966–974.
20. Ifergan I, Wosik K, Cayrol R, et al. Statins reduce human blood-brain barrier permeability and restrict leukocyte migration: relevance to multiple sclerosis. *Ann Neurol* 2006;60:45–55.
21. Erdos B, Snipes JA, Tulbert CD, Katakam P, Miller AW, Busija DW. Rosuvastatin improves cerebrovascular function in Zucker obese rats by inhibiting NAD(P)H oxidase-dependent superoxide production. *Am J Physiol Heart Circ Physiol* 2006;290:H1264–H1270.
22. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) investigators. *Circulation* 1999;100:230–235.
23. Gensini GF, Gori AM, Dilaghi B, et al. Effect of atorvastatin on circulating hsCRP concentrations: A sub-study of the Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration (ACTFAST) study. *Int J Cardiol* 2009 Feb 11. [Epub ahead of print].
24. Ridker PM, Danielson E, Fonseca FA, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet* 2009;373:1175–1182.
25. Tapia-Perez JH, Sanchez-Aguilar M, Torres-Corzo JG, et al. Effect of rosuvastatin on amnesia and disorientation after traumatic brain injury (NCT003229758). *J Neurotrauma* 2008;25:1011–1017.
26. Vink R, Nimmo AJ. Multifunctional drugs for head injury. *Neurotherapeutics* 2009;6:28–42.
27. Zacco A, Togo J, Spence K, et al. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors protect cortical neurons from excitotoxicity. *J Neurosci* 2003;23:11104–11111.
28. Ponce J, de la Ossa NP, Hurtado O, et al. Simvastatin reduces the association of NMDA receptors to lipid rafts: a cholesterol-mediated effect in neuroprotection. *Stroke* 2008;39:1269–1275.
29. Dolga AM, Nijholt IM, Ostroveanu A, Ten Bosch Q, Luiten PG, Eisel UL. Lovastatin induces neuroprotection through tumor necrosis factor receptor 2 signaling pathways. *J Alzheimers Dis* 2008;13:111–122.
30. Lewelt W, Jenkins LW, Miller JD. Autoregulation of cerebral blood flow after experimental fluid percussion injury of the brain. *J Neurosurg* 1980;53:500–511.
31. Wei EP, Dietrich WD, Povlishock JT, Navari RM, Kontos HA. Functional, morphological, and metabolic abnormalities of the cerebral microcirculation after concussive brain injury in cats. *Circ Res* 1980;46:37–47.
32. Klauber MR, Marshall LF, Luerssen TG, Frankowski R, Tabaddor K, Eisenberg HM. Determinants of head injury mortality: importance of the low risk patient. *Neurosurgery* 1989;24:31–36.
33. Murray GD, Butcher I, McHugh GS, et al. Multivariable prognostic analysis in traumatic brain injury: results from the IMPACT study. *J Neurotrauma* 2007;24:329–337.
34. Williams JK, Sukhova GK, Herrington DM, Libby P. Pravastatin has cholesterol-lowering independent effects on the artery wall of atherosclerotic monkeys. *J Am Coll Cardiol* 1998;31:684–691.
35. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. *Circulation* 1997;95:1126–1131.
36. Amin-Hanjani S, Stagliano NE, Yamada M, Huang PL, Liao JK, Moskowitz MA. Mevastatin, an HMG-CoA reductase inhibitor, reduces stroke damage and upregulates endothelial nitric oxide synthase in mice. *Stroke* 2001;32:980–986.
37. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 1998;97:1129–1135.
38. Wang H, Durham L, Dawson H, et al. An apolipoprotein E-based therapeutic improves outcome and reduces Alzheimer's disease pathology following closed head injury: evidence of pharmacogenomic interaction. *Neuroscience* 2007;144:1324–1333.
39. McGirt MJ, Woodworth GF, Pradilla G, et al. Galbraith Award: 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. *Clin Neurosurg* 2005;52:371–378.
40. Lu D, Mahmood A, Goussev A, Qu C, Zhang ZG, Chopp M. Delayed thrombolysis after traumatic brain injury in rats. *J Neurotrauma* 2004;21:1756–1766.
41. Lu D, Mahmood A, Goussev A, et al. Atorvastatin reduction of intravascular thrombosis, increase in cerebral microvascular patency and integrity, and enhancement of spatial learning in rats subjected to traumatic brain injury. *J Neurosurg* 2004;101:813–821.
42. Lynch JR, Wang H, McGirt MJ, et al. Simvastatin reduces vasospasm after aneurysmal subarachnoid hemorrhage: results of a pilot randomized clinical trial. *Stroke* 2005;36:2024–2026.
43. Qu C, Lu D, Goussev A, Schallert T, Mahmood A, Chopp M. Effect of atorvastatin on spatial memory, neuronal survival, and vascular density in female rats after traumatic brain injury. *J Neurosurg* 2005;103:695–701.
44. Lu D, Qu C, Goussev A, et al. Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. *J Neurotrauma* 2007;24:1132–1146.
45. Wu H, Lu D, Jiang H, et al. Increase in phosphorylation of Akt and its downstream signaling targets and suppression of apoptosis by simvastatin after traumatic brain injury. *J Neurosurg* 2008;109:691–698.
46. Wu H, Lu D, Jiang H, et al. Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. *J Neurotrauma* 2008;25:130–139.
47. Chen J, Zhang C, Jiang H, et al. Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. *J Cereb Blood Flow Metab* 2005;25:281–290.
48. Wang L, Gang Zhang Z, Lan Zhang R, Chopp M. Activation of the PI3-K/Akt pathway mediates cGMP enhanced neurogenesis in the adult progenitor cells derived from the subventricular zone. *J Cereb Blood Flow Metab* 2005;25:1150–1158.
49. Horwood JM, Dufour F, Laroche S, Davis S. Signalling mechanisms mediated by the phosphoinositide 3-kinase/Akt cascade in synaptic plasticity and memory in the rat. *Eur J Neurosci* 2006;23:3375–3384.
50. Chen J, Zhang ZG, Li Y, et al. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. *Ann Neurol* 2003;53:743–751.

51. Weis M, Heeschen C, Glassford AJ, Cooke JP. Statins have biphasic effects on angiogenesis. *Circulation* 2002;105:739–745.
52. Hixon ML, Muro-Cacho C, Wagner MW, et al. Akt1/PKB upregulation leads to vascular smooth muscle cell hypertrophy and polyploidization. *J Clin Invest* 2000;106:1011–1020.
53. Narayan RK, Michel ME, Ansell B, et al. Clinical trials in head injury. *J Neurotrauma* 2002;19:503–557.
54. Rinder L. “Concussive response” and intracranial pressure changes at sudden extradural fluid volume input in rabbits. *Acta Physiol Scand* 1969;76:352–360.
55. Wang H, Gao J, Lassiter TF, et al. Levetiracetam is neuroprotective in murine models of closed head injury and subarachnoid hemorrhage. *Neurocrit Care* 2006;5:71–78.
56. Lynch JR, Pineda JA, Morgan D, et al. Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Ann Neurol* 2002;51:113–117.
57. Lynch JR, Wang H, Mace B, et al. A novel therapeutic derived from apolipoprotein E reduces brain inflammation and improves outcome after closed head injury. *Exp Neurol* 2005;192:109–116.
58. Zohar O, Schreiber S, Getslev V, Schwartz JP, Mullins PG, Pick CG. Closed-head minimal traumatic brain injury produces long-term cognitive deficits in mice. *Neuroscience* 2003;118:949–955.
59. Mahmood A, Goussev A, Lu D, et al. Long-lasting benefits after treatment of traumatic brain injury (TBI) in rats with combination therapy of marrow stromal cells (MSCs) and simvastatin. *J Neurotrauma* 2008;25:1441–1447.
60. McGirt MJ, Lynch JR, Parra A, et al. Simvastatin increases endothelial nitric oxide synthase and ameliorates cerebral vasospasm resulting from subarachnoid hemorrhage. *Stroke* 2002;33:2950–2956.
61. Cheng G, Wei L, Zhi-Dan S, Shi-Guang Z, Xiang-Zhen L. Atorvastatin ameliorates cerebral vasospasm and early brain injury after subarachnoid hemorrhage and inhibits caspase-dependent apoptosis pathway. *BMC Neurosci* 2009;10:7.
62. Sugawara T, Ayer R, Jadhav V, Chen W, Tsubokawa T, Zhang JH. Simvastatin attenuation of cerebral vasospasm after subarachnoid hemorrhage in rats via increased phosphorylation of Akt and endothelial nitric oxide synthase. *J Neurosci Res* 2008;86:3635–3643.
63. McGirt MJ, Pradilla G, Legnani FG, et al. Systemic administration of simvastatin after the onset of experimental subarachnoid hemorrhage attenuates cerebral vasospasm. *Neurosurgery* 2006;58:945–951.
64. Bulsara KR, Coates JR, Agrawal VK, et al. Effect of combined simvastatin and cyclosporine compared with simvastatin alone on cerebral vasospasm after subarachnoid hemorrhage in a canine model. *Neurosurg Focus* 2006;21:E11.
65. Seyfried D, Han Y, Lu D, Chen J, Bydon A, Chopp M. Improvement in neurological outcome after administration of atorvastatin following experimental intracerebral hemorrhage in rats. *J Neurosurg* 2004;101:104–107.
66. Jung KH, Chu K, Jeong SW, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. *Stroke* 2004;35:1744–1749.
67. Prinz V, Laufs J, Gertz K, et al. Intravenous rosuvastatin for acute stroke treatment: an animal study. *Stroke* 2008;39:433–438.
68. Mayanagi K, Katakam PV, Gaspar T, Domoki F, Busija DW. Acute treatment with rosuvastatin protects insulin resistant (C57BL/6J ob/ob) mice against transient cerebral ischemia. *J Cereb Blood Flow Metab* 2008;28:1927–1935.
69. Sironi L, Cimino M, Guerrini U, et al. Treatment with statins after induction of focal ischemia in rats reduces the extent of brain damage. *Arterioscler Thromb Vasc Biol* 2003;23:322–327.
70. Naval NS, Abdelhak TA, Zeballos P, Urrunaga N, Mirski MA, Carhuapoma JR. Prior statin use reduces mortality in intracerebral hemorrhage. *Neurocrit Care* 2008;8:6–12.
71. Naval NS, Abdelhak TA, Urrunaga N, Zeballos P, Mirski MA, Carhuapoma JR. An association of prior statin use with decreased perihematomal edema. *Neurocrit Care* 2008;8:13–18.
72. Leker RR, Khoury ST, Rafaeli G, Shwartz R, Eichel R, Tanne D. Prior use of statins improves outcome in patients with intracerebral hemorrhage: prospective data from the National Acute Stroke Israeli Surveys (NASIS). *Stroke* 2009;40:2581–2584.
73. FitzMaurice E, Wendell L, Snider R, et al. Effect of statins on intracerebral hemorrhage outcome and recurrence. *Stroke* 2008;39:2151–2154.
74. Elkind MS, Sacco RL, MacArthur RB, et al. The Neuroprotection with Statin Therapy for Acute Recovery Trial (NeuSTART): an adaptive design phase I dose-escalation study of high-dose lovastatin in acute ischemic stroke. *Int J Stroke* 2008;3:210–218.
75. Tseng MY, Czosnyka M, Richards H, Pickard JD, Kirkpatrick PJ. Effects of acute treatment with pravastatin on cerebral vasospasm, autoregulation, and delayed ischemic deficits after aneurysmal subarachnoid hemorrhage: a phase II randomized placebo-controlled trial. *Stroke* 2005;36:1627–1632.
76. Chou SH, Smith EE, Badjatia N, et al. A randomized, double-blind, placebo-controlled pilot study of simvastatin in aneurysmal subarachnoid hemorrhage. *Stroke* 2008;39:2891–2893.
77. Vergouwen MD, Meijers JC, Geskus RB, et al. Biologic effects of simvastatin in patients with aneurysmal subarachnoid hemorrhage: a double-blind, placebo-controlled randomized trial. *J Cereb Blood Flow Metab* 2009;29:1444–1453.
78. Kerz T, Victor A, Beyer C, Trapp I, Heid F, Reisch R. A case control study of statin and magnesium administration in patients after aneurysmal subarachnoid hemorrhage: incidence of delayed cerebral ischemia and mortality. *Neurol Res* 2008;30:893–897.
79. Kramer AH, Gurka MJ, Nathan B, Dumont AS, Kassell NF, Bleck TP. Statin use was not associated with less vasospasm or improved outcome after subarachnoid hemorrhage. *Neurosurgery* 2008;62:422–430.
80. McGirt MJ, Garces Ambrossi GL, Huang J, Tamargo RJ. Simvastatin for the prevention of symptomatic cerebral vasospasm following aneurysmal subarachnoid hemorrhage: a single-institution prospective cohort study. *J Neurosurg* 2009;110:968–974.
81. Kern M, Lam MM, Knuckey NW, Lind CR. Statins may not protect against vasospasm in subarachnoid haemorrhage. *J Clin Neurosci* 2009;16:527–530.
82. Tseng MY, Hutchinson PJ, Turner CL, et al. Biological effects of acute pravastatin treatment in patients after aneurysmal subarachnoid hemorrhage: a double-blind, placebo-controlled trial. *J Neurosurg* 2007;107:1092–1100.
83. STASH Trial: SimvaSTatin in Aneurysmal Subarachnoid Hemorrhage. Available at: www.stashtrial.com. Accessed August 18, 2009.
84. Margulies S, Hicks R. Combination therapies for traumatic brain injury: prospective considerations. *J Neurotrauma* 2009;26:925–939.
85. Besson VC, Chen XR, Plotkine M, Marchand-Verrecchia C. Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, exerts neuroprotective effects in traumatic brain injury. *Neurosci Lett* 2005;388:7–12.
86. Balduini W, Mazzoni E, Carloni S, et al. Prophylactic but not delayed administration of simvastatin protects against long-lasting cognitive and morphological consequences of neonatal hypoxic-ischemic brain injury, reduces interleukin-1beta and tumor necrosis factor-alpha mRNA induction, and does not affect endothelial nitric oxide synthase expression. *Stroke* 2003;34:2007–2012.
87. Saheki A, Terasaki T, Tamai I, Tsuji A. In vivo and in vitro blood-brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. *Pharm Res* 1994;11:305–311.
88. Miron VE, Zehntner SP, Kuhlmann T, et al. Statin therapy inhibits remyelination in the central nervous system. *Am J Pathol* 2009;174:1880–1890.
89. Amarenco P, Bogousslavsky J, Callahan A, 3rd, et al. High-dose atorvastatin after stroke or transient ischemic attack. *N Engl J Med* 2006;355:549–559.
90. Bang OY, Saver JL, Liebeskind DS, et al. Cholesterol level and symptomatic hemorrhagic transformation after ischemic stroke thrombolysis. *Neurology* 2007;68:737–742.
91. Blanco M, Nombela F, Castellanos M, et al. Statin treatment withdrawal in ischemic stroke: a controlled randomized study. *Neurology* 2007;69:904–910.
92. Singhal AB, Topcuoglu MA, Dorer DJ, Ogilvy CS, Carter BS, Ko-

- roshetz WJ. SSRI and statin use increases the risk for vasospasm after subarachnoid hemorrhage. *Neurology* 2005;64:1008–1013.
93. Menon DK. Unique challenges in clinical trials in traumatic brain injury. *Crit Care Med* 2009;37:S129–135.
94. Tseng MY, Hutchinson PJ, Czosnyka M, Richards H, Pickard JD, Kirkpatrick PJ. Effects of acute pravastatin treatment on intensity of rescue therapy, length of inpatient stay, and 6-month outcome in patients after aneurysmal subarachnoid hemorrhage. *Stroke* 2007;38:1545–1550.
95. Montaner J, Chacon P, Krupinski J, et al. Simvastatin in the acute phase of ischemic stroke: a safety and efficacy pilot trial. *Eur J Neurol* 2008;15:82–90.