The Neuroprotective Effects of Simvastatin on High Cholesterol Following Traumatic Brain Injury in Rats

Arng Jack Chong[1], Sher-Wei Lim[2,3], Yao-lin Lee[1], Chung-Ching Chio[1], Chin-Hung Chang[1], Jinn-Rung Kuo[1,5], Che-Chuan Wang[1,4,5]

■ BACKGROUND: **High cholesterol has been correlated with a greater risk of cerebrovascular diseases. Whether pre-existing high cholesterol exacerbates traumatic brain injury (TBI), and whether treatment with the cholesterol-lowering agent simvastatin has neuroprotective effects, especially anti-neuroinflammatory effects, after TBI are not well investigated.**

■ METHODS: **Five-week-old male Sprague–Dawley rats were fed a high-fat diet for 8 weeks to induce hypercholesterolemia. Anesthetized male Sprague–Dawley rats were divided into 5 groups, including the sham-operated control, TBI control, and TBI with simvastatin treatment (4 mg/kg, 10 mg/kg, or 20 mg/kg) groups. Simvastatin was intraperitoneally injected at 0, 24, and 48 hours after TBI. Motor function was measured using an inclined plane. Neuronal apoptosis (maker Neu-N, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling), tumor necrosis factor-α expression in microglia (marker OX42) and astrocytes (marker glial fibrillary acidic protein), and Tumor necrosis factor-alpha receptor (TNFR) 1 and TNFR2 expression in neurons in the ischemic cortex were investigated using an immunofluorescence assay. All of the parameters were measured on the third day after TBI.**

■ RESULTS: **TBI significantly increased the serum levels of cholesterol. The TBI-induced motor deficit was significantly attenuated by 4, 10, and 20 mg/kg simvastatin therapy on the third day after TBI. TBI-induced neuronal TNFR1 activation and apoptosis, as well as tumor necrosis factor-α expression in astrocytes in the ischemic cortex, were significantly attenuated by simvastatin, particularly when 20 mg/kg was administered. Simultaneously, the serum cholesterol remained high despite simvastatin treatment.**

■ CONCLUSIONS: **The neuroprotection effects of simvastatin on the pre-existing hypercholesterolemia during TBI in rats may be related to its anti-neuroinflammatory effects but not to its cholesterol-lowing effects.**

INTRODUCTION

High cholesterol is a common symptom in the general population and has been defined as an increase in fasting serum cholesterol.[1] High cholesterol is well known to increase the risk of cerebrovascular diseases.[2] Thirumangalakudi et al.[3] indicated that a high-cholesterol diet induces neuroinflammation characterized by glial activation and proinflammatory cytokines and cytokine expression in a model of dementia. Kay et al.[4] demonstrated that, following traumatic brain injury (TBI), patients show an increase in cholesterol in the cerebrospinal fluid (CSF) because the brain is very cholesterol-rich. However, whether

Key words
- Apoptosis, tumor necrosis factor-alpha
- Cholesterol
- Fluid percussion injury
- Simvastatin
- Tumor necrosis factor-alpha receptor

Abbreviations and Acronyms
CSF: Cerebrospinal fluid
DAPI: 4′,6-diamidino-2-phenylindole
GFAP: Glial fibrillary acidic protein
i.m.: Intramuscularly
TBI: Traumatic brain injury
TNF-α: Tumor necrosis factor-alpha
TNFR1/TNFR2: Tumor necrosis factor-alpha receptor 1/2
TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling

From the [1]Department of Neurosurgery, Chi-Mei Medical Center, Tainan; [2]Department of Neurosurgery, Chi-Mei Medical Center, Chiali, Tainan; [3]Department of Nursing, Min-Hwei College of Health Care Management, Tainan; [4]Department of General Education, Southern Taiwan University of Science and Technology, Tainan; and [5]Department of Medical Research Chi-Mei Medical Center, Tainan, Taiwan

To whom correspondence should be addressed: Jinn-Rung Kuo, Ph.D. [E-mail: kuojinnrung@gmail.com]

Arng Jack Chong and Sher-Wei Lim contributed equally to this work.

Jinn-Rung Kuo and Che-Chuan Wang contributed equally to this work.

Citation: World Neurosurg. (2019) 132:e99-e108.
https://doi.org/10.1016/j.wneu.2019.08.250

*Journal homepage: **www.journals.elsevier.com/world-neurosurgery***

*Available online: **www.sciencedirect.com***

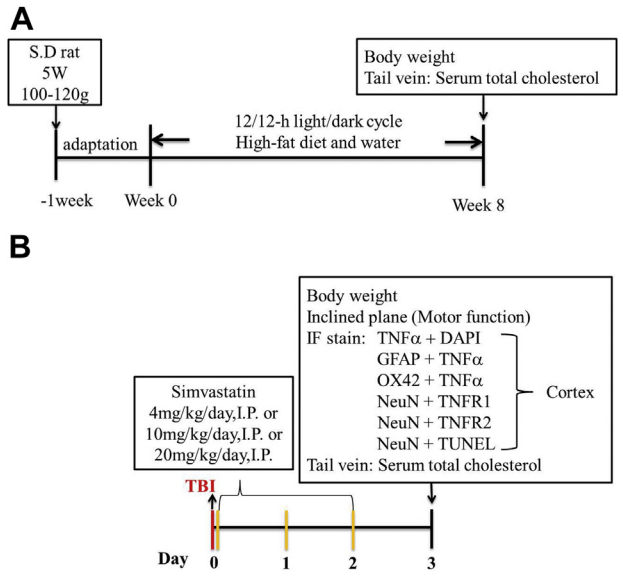

Figure 1. The overall experimental protocol in the current study. SD, Sprague–Dawley; I.P., intraperitoneally; TNF-α, tumor necrosis factor-α; DAPI, 4′,6-diamidino-2-phenylindole; GFAP, glial fibrillary acidic protein; TNFR1, tumor necrosis factor-alpha receptor 1; TNFR2, Tumor necrosis factor-alpha receptor 2; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling.

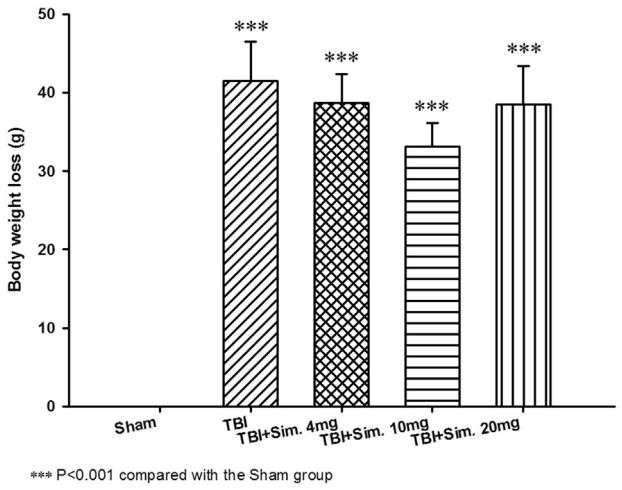

Figure 2. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced loss of body weight 72 hours after TBI in the rats with pre-existing hypercholesterol. ***$P < 0.001$ ($n = 6$ in each group).

pre-existing high cholesterol exacerbates TBI in experimental rats is not well investigated.

Astrocyte and microglia activation are markers of neuroinflammation that result in the release of tumor necrosis factor-alpha (TNF-α), and these markers have been observed in the brain following TBI.[5,6] Several studies have shown TNF-α transduces death and survival signaling through its cognate receptors, tumor necrosis factor-alpha receptor 1/2 (TNFR1/TNFR2), in neurons.[7,8] Therefore, neuroinflammation may play a key role in exacerbating the outcome of TBI. However, at this time, the effects of pre-existing high cholesterol in the rat (induced by feeding with a high cholesterol diet) before TBI are not well reported.

Simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor and a cholesterol-lowering agent, is commonly applied in clinical settings.[9] Simvastatin has been found to be neuroprotective in both transient and permanent experimental TBI via its anti-inflammatory effects.[10,11] However, some reports have indicated that statins were potentially neuroprotective independent of their effects on serum cholesterol.[12,13] Therefore, it is worth investigating the neuroprotective mechanisms of simvastatin by using a specific rat model of high cholesterol in TBI.

In the current study, we hypothesized that TBI may induce the development of greater cholesterol and neuroinflammatory events.

Table 1. Characteristic of Male SD Rats after 8 Weeks of a Normal Diet or High-Fat Diet

	Normal Diet	High-Fat Diet
Body weight, g	453 ± 9.49	489 ± 10.85*
Serum total cholesterol, mmol/L	1.44 ± 0.06	1.79 ± 0.05†

SD, Sprague–Dawley.
*Significant difference, $P < 0.05$.
†Significant difference, $P < 0.001$.

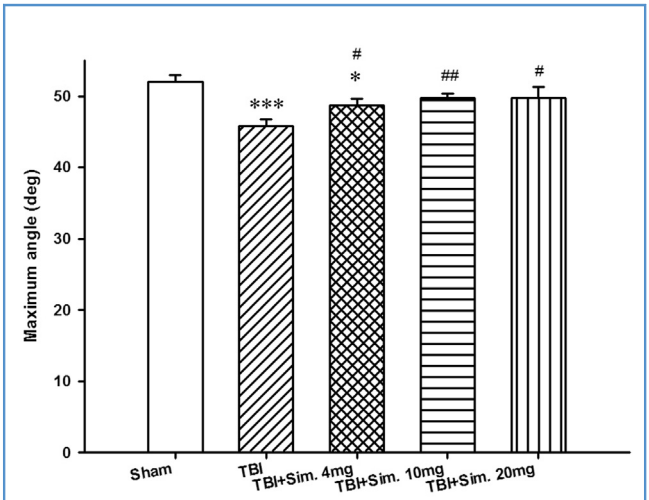

Figure 3. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced motor deficits 72 hours after TBI in the rats with pre-existing hypercholesterol. *$P < 0.05$, ***$P < 0.001$, #$P < 0.05$, and ##$P < 0.01$ ($n = 6$ in each group).

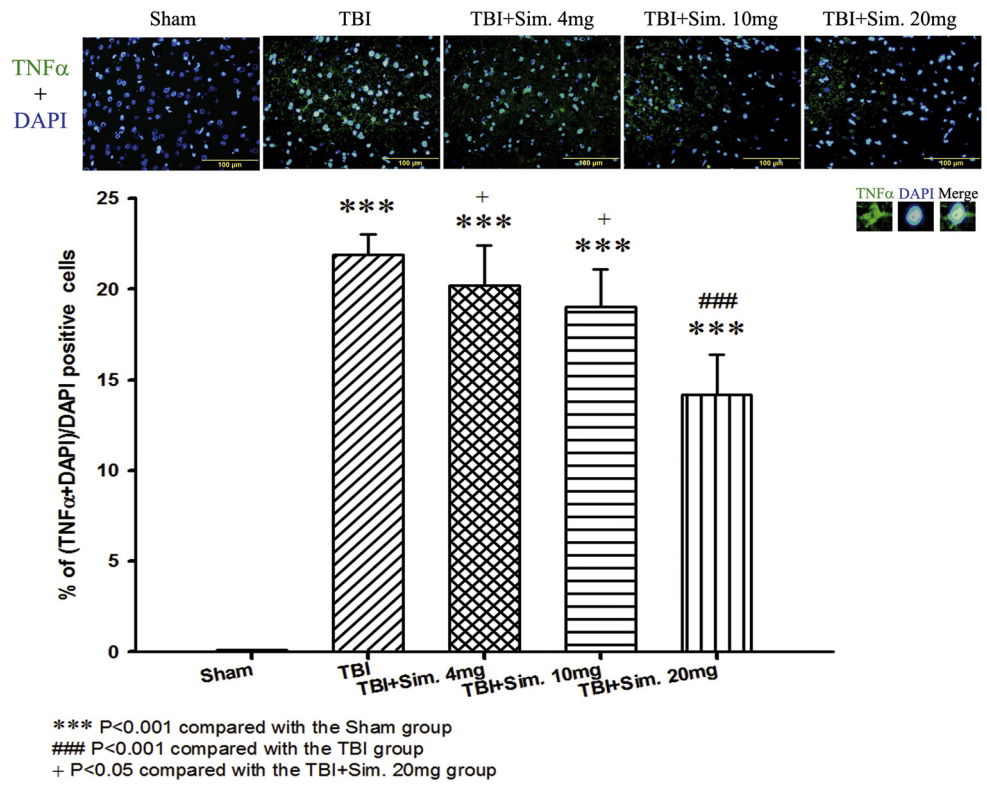

Figure 4. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced TNF-α expression 72 hours following TBI (DAPI + TNF-α marker) in the peri-lesioned cortex. #P <0.05, ##P < 0.01, ###P < 0.001, and ***P < 0.001 (n = 6 in each group). DAPI, 4′,6-diamidino-2-phenylindole; TNF-α, tumor necrosis factor-α.

We also hypothesize that the neuroprotective effects of simvastatin after TBI may relate to its anti-inflammatory effects but not to its cholesterol-lowering effects. To test this hypothesis, we investigate regional microglia, astrocyte activation, TNF-α, TNFR1/TNFR2 expression, neuronal apoptosis in the brain, peripheral total cholesterol concentration, and functional outcomes on the third day after TBI. We believe our results will elucidate the possible neuroprotective mechanisms of simvastatin in the high cholesterol TBI model.

MATERIALS AND METHODS

Experimental Design

The overall experimental protocol is shown in Figure 1.

Induced High-Cholesterol Rat Model Methods

Five-week-old male Sprague–Dawley rats were fed a high fat diet containing 34.9% (wt/wt) fat and 301 ppm cholesterol (58Y1; TestDiet, Richmond, Indiana, USA) for 8 weeks. The animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act. At the end of the treatment period, total serum cholesterol and body weight were measured. The blood samples were analyzed using an auto-analyzer (Quik-Lab, Elkhart, Indiana, USA).

Traumatic Brain Injury

Animals were anesthetized and injected intraperitoneally with a mixture of ketamine (44 mg/kg, intramuscularly [i.m.]; Nang Kuang Pharmaceutical, Tainan, Taiwan), atropine (0.02633 mg/kg, i.m.; Sintong Chemical Ind. Co., Taipei City, Taiwan), and Rompun (6.77 mg/kg, i.m.; Bayer, Leverkusen, Germany). A craniectomy with a 2-mm radius, 4 mm from bregma, and 3 mm from sagittal sutures in the right parietal cortex was performed using a stereotaxic frame. Then, the fluid percussion device (VCU Biomedical Engineering, Richmond, Virginia, USA) was connected, and the brain was injured with a 2.2 atm, 25-millisecond percussion, which produces moderate-severity brain trauma. The detailed procedures are available in a previous article.[14]

Treatment Intervention

Using the random number table, we numbered the rats in random order and assigned them to different study groups: sham operated (n = 6); TBI treated with vehicle, dimethylsulfoxide (4%, 1 mL/kg,

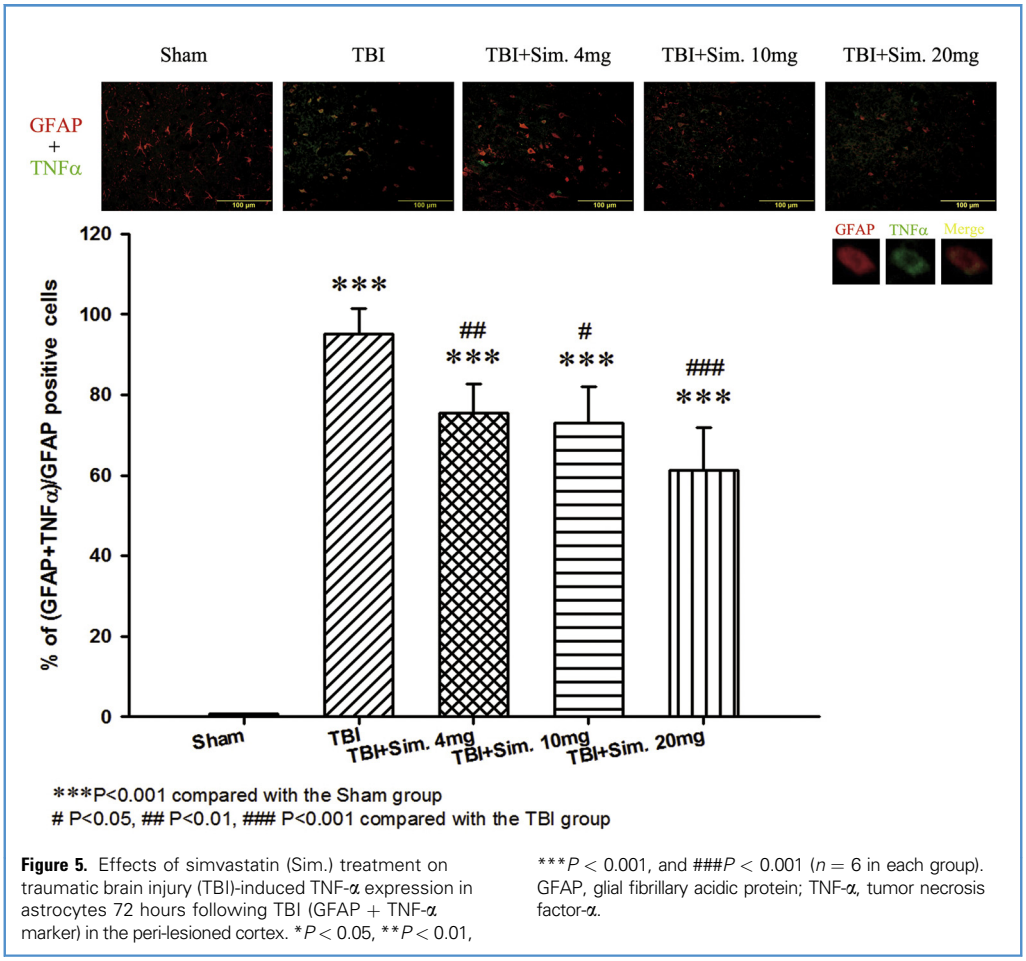

Figure 5. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced TNF-α expression in astrocytes 72 hours following TBI (GFAP + TNF-α marker) in the peri-lesioned cortex. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $\#\#\#P < 0.001$ ($n = 6$ in each group). GFAP, glial fibrillary acidic protein; TNF-α, tumor necrosis factor-α.

injected intraperitoneally, K42088831, vehicle; Merck, Darmstadt, Germany); and TBI + simvastatin-treated (4 mg/kg or 10 mg/kg or 20 mg/kg, dissolved with dimethylsulfoxide, injected intraperitoneally; U.S. Pharmacopeia [North Bethesda, Maryland, USA]; n = 6 for each dose). Simvastatin or vehicle was injected 24 and 48 hours after TBI. All tests were performed with the investigators blind to the study groups, which were revealed only at the conclusion of analysis.

Cholesterol Measurement

The blood was allowed to flow into the tube by capillary action from the tail vein. After collecting the blood (1 mL), we ensured that there was no more bleeding, and the rats were returned to their cages. Blood samples were centrifuged 3500 rpm (1962*g*) for 15 minutes at 4°C to separate the serum and blood cells. The cholesterol concentration was measured with an enzymatic method using an Architect C8000 machine (Abbott Diagnostics, Chicago, Illinois, USA).

Motor Function Test

An inclined plane is commonly used to measure limb strength.[15] The animals were placed facing right and then left, perpendicular to the slope of a 20 × 20-cm buffer ribbed surface of an inclined plane initially positioned at a 55° angle. To determine the maximal angle at which an animal could remain on the incline plane, the angle was increased or decreased in 5° increments. Motor deficit measurements were conducted with left- and right-side maximal angles on the third day after TBI.

Immunofluorescence Staining

Adjacent 6-μm sections corresponding to coronal coordinates 0.20—0.70 mm anterior to the bregma were incubated in 2 mol/L HCl for 30 minutes, rinsed in 0.1 mol/L boric acid (pH 8.5) for 3 minutes at room temperature, and then incubated with primary antibodies in phosphate-buffered saline containing 0.5% normal bovine serum at 4°C overnight. After being washed in phosphate-buffered saline, the sections were incubated with secondary antibodies for 1 hour at room temperature. The number of immunofluorescence assay—positive cells was calculated in 5 coronal sections corresponding to the peri-ischemic cortex (400× magnification) and expressed as the mean number of positive cells in all 5 sections from each rat using computerized planimetry (Image-Pro Plus Media Cybernetics, Inc., Rockville, Maryland, USA).

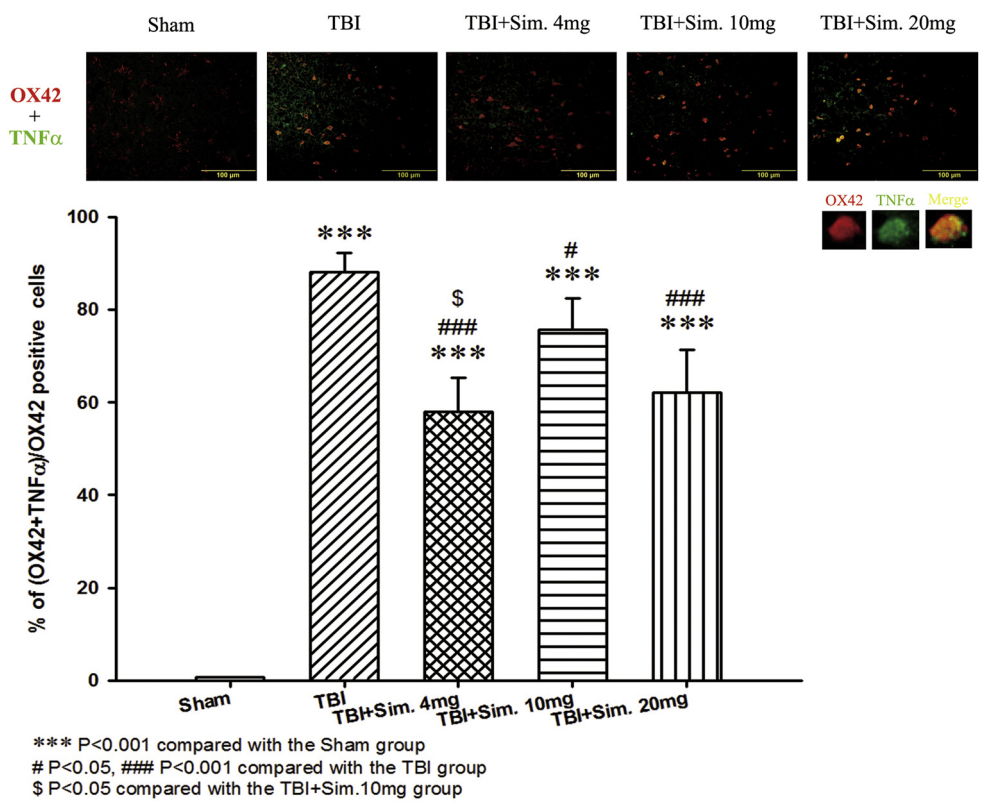

Figure 6. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced TNF-α expression in microglia 72 hours following TBI (OX42 + TNF-α marker) in the peri-lesioned cortex. #$P < 0.05$, ###$P < 0.001$, and ***$P < 0.001$ ($n = 6$ in each group). TNF-α, tumor necrosis factor-α.

Apoptotic Assay in Neuronal Cells in the Cortex Using Immunofluorescence Staining

Neuronal apoptotic cells were identified at 72 hours after TBI by staining with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL).[16] These procedures followed those described previously.[14] The percentage of TUNEL-positive and Neu-N—positive cells was calculated in 5 coronal sections from each rat in the samples and summed using computerized planimetry (PC-based image tools software). The following antibodies were used in this study: monoclonal mouse anti-NeuN antibody (NeuN, MAB377; MilliporeSigma, Burlington, Massachusetts, USA) at 1:100 dilution, which was detected with an Alexa-Fluor 568 anti-mouse (IgG) antibody (A11031; Invitrogen, Carlsbad, California, USA) at 1:400 dilution.

The activated microglia and astrocytes were evaluated on the third day after TBI by detecting OX42-and glial fibrillary acidic protein (GFAP)-positive cells using an immunofluorescent assay.[17] The TNF-α expression in the activated microglia or astrocytes were evaluated on the third day after TBI by detecting OX42 or GFAP within TNF-α—positive cells using an immunofluorescent assay. The percentage of OX42 or GFAP and TNF-α—positive cells in the samples were measured in each section and summed using computerized planimetry (PC-based image tools software). The following antibodies were used in this study: a monoclonal mouse anti-OX42 antibody (ab1211, 1:200; Abcam, Cambridge, United Kingdom), monoclonal mouse anti-GFAP antibody (#3670, 1:400; Cell Signaling Technology Europe B.V, the Netherlands;), and polyclonal rabbit anti-TNF-α antibody (ab6671, 1:200; Abcam, Cambridge, United Kingdom); the antibodies were detected with an Alexa-Fluor 594 anti-mouse (IgG) antibody (ab150116, 1:400; Abcam, Cambridge, United Kingdom) and an Alexa-Fluor 488 anti-rabbit (IgG) antibody (ab150077, 1:400; Abcam, Cambridge, United Kingdom), respectively.

TNFR1 and TNFR2 Expression Assay in Neuronal Cells

TNFR1 and TNFR2 expression in neuronal cells was evaluated on the third day after TBI by detecting TNFR1-and TNFR2-positive cells using an immunofluorescent assay. The following antibodies were used in this study: rabbit polyclonal anti-TNFR1 antibody (ab19139; Abcam, Cambridge, Massachusetts, USA) at a 1:600 dilution detected with an Alexa-Fluo 488 anti-rabbit (IgG) antibody (ab150077; Abcam, Cambridge, Massachusetts, USA) at a 1:400 dilution, and a rabbit monoclonal anti-TNFR2 antibody (ab109322; Abcam, Cambridge, Massachusetts, USA) at a 1:100 dilution detected with an Alexa-Fluor 488 anti-rabbit (IgG)

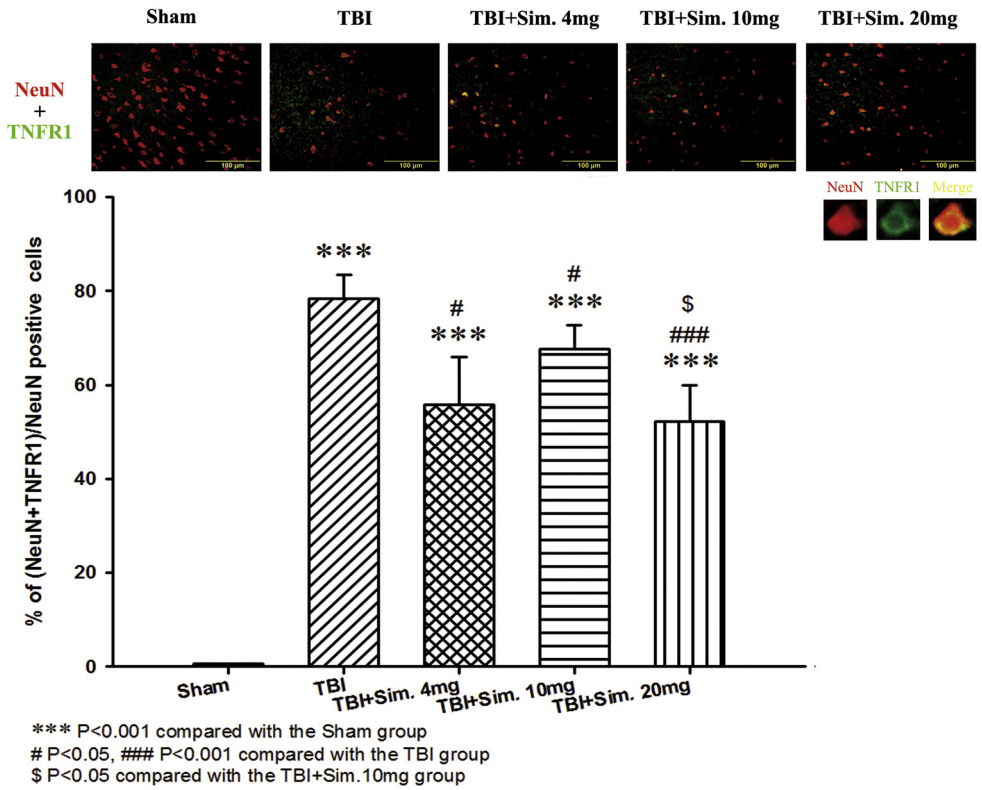

Figure 7. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced neuronal tumor necrosis factor-alpha receptor 1 (TNFR1) 72 hours following TBI. Simvastatin significantly attenuated TBI-induced neuronal TNFR1 and TNFR2 in the peri-lesioned cortex. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, and ***$P < 0.001$ ($n = 6$ in each group).

antibody (ab150077; Abcam, Cambridge, Massachusetts, USA) at a 1:400 dilution.

Statistical Analysis

The results are expressed as the means ±standard deviation of the means for the experiments. A 2-way analysis of variance for repeated measurements (in the same animals) was used for factorial experiments, and the Dunnett test was used for post-hoc multiple comparisons among means. A value of $P < 0.05$ was considered significant. All of the data were analyzed using SigmaPlot, version 10.0 for Windows (Systat Software, San Jose, California, USA).

RESULTS

Induced High-Cholesterol Rat Model

Table 1 shows that the high cholesterol rat was successfully induced. The body weights of the high-cholesterol rats were significantly increased compared with rats on a normal chow diet (453 ± 9.49 g vs. 515 ± 13.6 g; $P < 0.001$). At the same time, the serum cholesterol of the high-fat diet rats was significantly increased compared with the rats receiving a normal-chow diet (1.44 ± 0.06 mmol/L vs. 1.81 ± 0.06; $P < 0.001$).

Significant Loss in Body Weight on the Third Day in the TBI and Simvastatin-Treated TBI Groups

On the third day after TBI, the loss in body weight was significantly increased in the TBI and simvastatin-treated TBI rats compared with those of the sham controls (***$P < 0.001$, Figure 2). However, no significant difference in the loss of body weight was observed on the third day in the TBI vehicle-treated rats compared with each of the simvastatin-treated TBI groups.

Post-TBI Treatment with Simvastatin Significantly Improved Motor Function Outcomes

The maximal grip angle, measuring the muscle strength of the 4 limbs of the rats, on the third day after TBI was significantly lower compared with sham controls (***$P < 0.001$). TBI-induced motor dysfunction was significantly improved by 4, 10, and 20 mg/kg simvastatin treatment, *$P < 0.05$, ##$P < 0.01$, and #$P < 0.05$, respectively, compared with the TBI vehicle-treated rats (Figure 3).

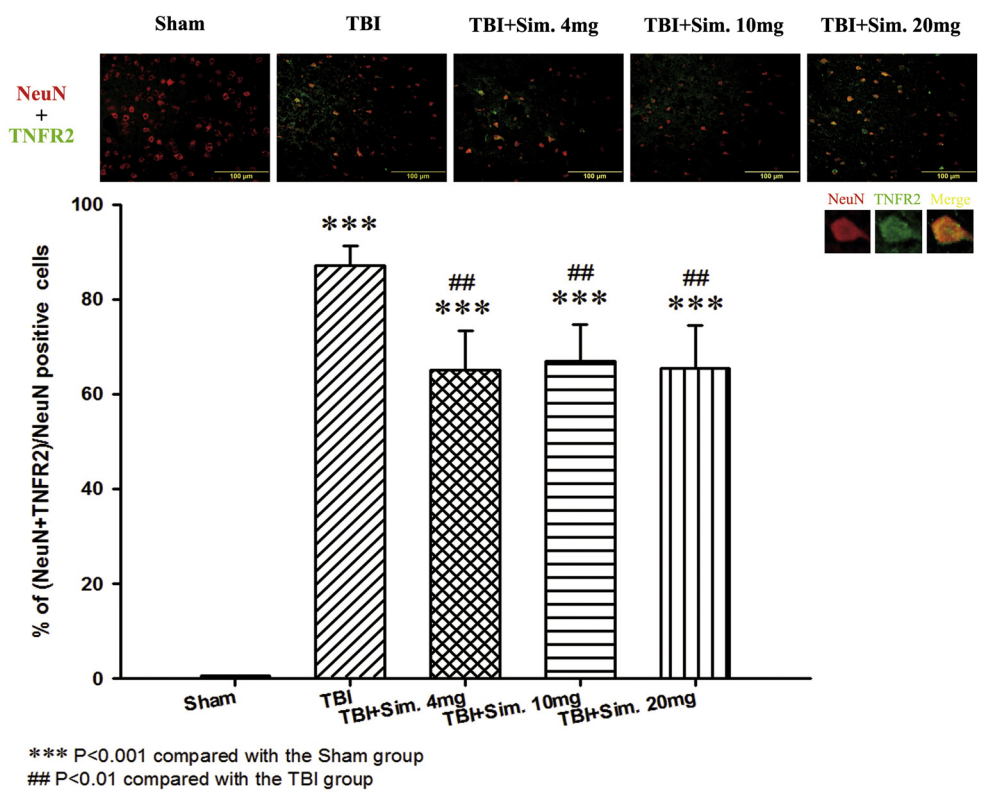

Figure 8. Effects of simvastatin treatment on traumatic brain injury (TBI)-induced neuronal tumor necrosis factor-alpha receptor 2 (TNFR2) 72 hours following TBI. Simvastatin (Sim.) significantly attenuated TBI-induced neuronal TNFR1 and TNFR2 in the peri-lesioned cortex. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, and ***$P < 0.001$ ($n = 6$ in each group).

Simvastatin Significantly Decreased TBI-Induced TNF-α Expression in Astrocytes and Microglia in the Peri-Lesioned Cortex on Day 3 after TBI

The TNF-α plus 4′,6-diamidino-2-phenylindole (DAPI) double staining showed that the percentage of TNF-α and DAPI-positive cells in the peri-lesioned cortex region of the TBI rats was significantly greater than that in the sham-operated rats (***P < 0.001). However, compared with the TBI vehicle-treated rats, the percentage of TBI-induced positive TNF-α in the DAPI-positive cells was significantly lower in the simvastatin-treated rats, particularly in the 20 mg/kg group (###P < 0.001, Figure 4).

GFAP or OX42 plus TNF-α double staining showed that the percentage of positive TNF-α in the activated astrocytes (Figure 5) and microglia (Figure 6) in the peri-lesioned cortex region of the TBI rats was significantly greater than in the sham-operated rats. However, compared with the TBI vehicle-treated rats, the percentage of TBI-induced positive TNF-α in the activated astrocytes and microglia was significantly lower in the simvastatin-treated rats, particularly in the 20 mg/kg group (###P < 0.001, Figures 5 and 6). These results support our hypothesis that anti-neuroinflammation is one mechanism of the neuroprotective effects of simvastatin.

Post-TBI Treatment with Simvastatin Significantly Improved the TBI-Induced TNFR1 and TNFR2 Expression in Neuronal Cells and Neuronal Apoptosis in the Peri-Lesioned Cortex

In the TNFR1 plus Neu-N–stained assay, the number of positive neuronal TNFR1 cells in the peri-lesioned cortex of the vehicle-treated rats was significantly increased compared with those in the sham controls (***P < 0.001, Figure 7). The TBI-induced increase in the percentage of neuronal TNFR1-positive cells was significantly attenuated by simvastatin therapy, particularly in the 20 mg/kg group (###P < 0.001, Figure 7).

Compared with the TBI vehicle-treated rats, the percentage of TBI-induced TNFR2 plus Neu-N–stained cells was significantly lower in the simvastatin-treated rats (##P < 0.05, Figure 8).

Using the TUNEL assay on the third day after TBI, we found that the percentage of apoptotic neuronal cells (Neu-N plus TUNEL staining assays) in the peri-lesioned cortex was significantly increased compared with that of the sham rats (***P < 0.001). Moreover, the percentage of positive apoptotic cells in rats with TBI was significantly reduced after simvastatin treatment, particularly in the 20 mg/kg group (###P < 0.001, Figure 9).

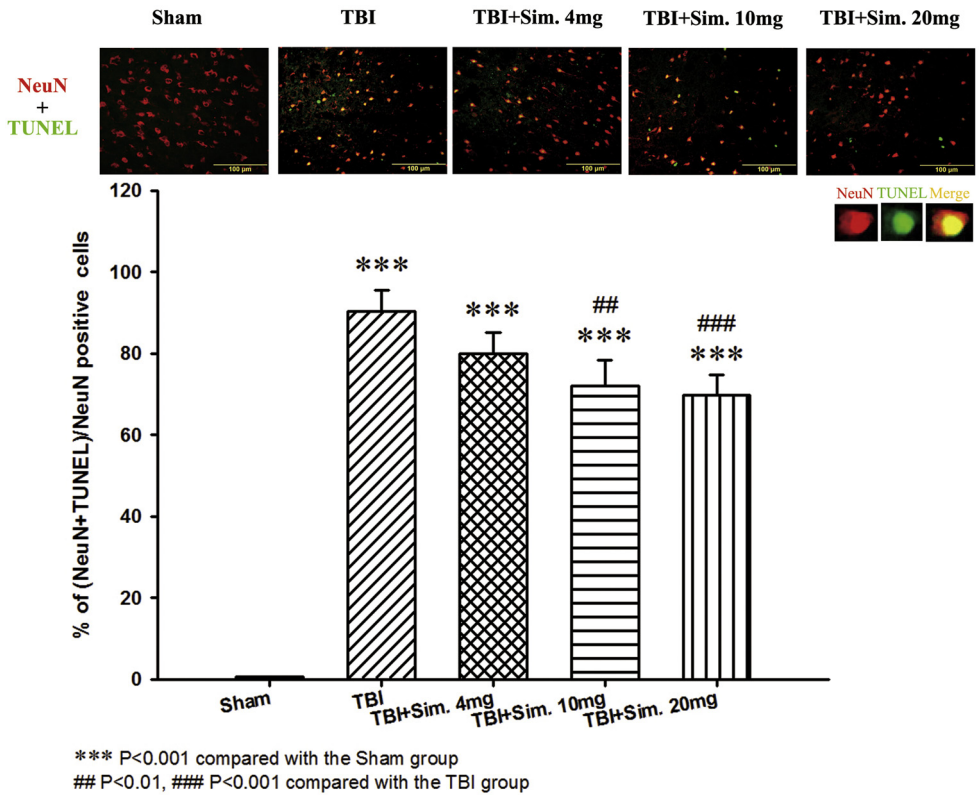

Figure 9. Effects of simvastatin (Sim.) treatment on traumatic brain injury (TBI)-induced neuronal apoptosis 72 hours following TBI. Simvastatin, particularly 20 mg/kg, significantly attenuated TBI-induced neuronal apoptosis (Neu-N + TUNEL) in the peri-lesioned cortex. $*P < 0.05$ and $***P < 0.001$ ($n = 6$ in each group). TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling.

Serum Cholesterol Remained High Despite Simvastatin Treatment After TBI

Figure 10 shows that on the third day following TBI, the serum cholesterol was significantly increased in the TBI vehicle-treated rats compared with that in the sham controls (*P < 0.05). Moreover, the serum cholesterol remained high despite simvastatin treatment at 4, 10, and 20 mg/kg after TBI.

DISCUSSION

In the current study, simvastatin, which permeates the blood–brain barrier and has clear pharmacokinetic activity,[18] was administered for 3 consecutive days after TBI in rats with pre-existing hypercholesterol. The findings showed that even though simvastatin did not lower the serum cholesterol in these rats, it attenuated the neuroinflammation in the peri-lesioned region and the motor deficits induced by TBI in rats with pre-existing hypercholesterol. To our knowledge, our study is the first to reveal neuroprotection associated with anti-neuroinflammation using simvastatin therapy in the pre-existing hypercholesterol TBI rat model.

Wang et al.[19] reported that simvastatin subcutaneously injected at 20 mg/kg/day for 3 days after TBI suppresses inflammatory cytokine mRNA expression in the parenchyma and diminishes hippocampal degeneration. In our previous study, we demonstrated that simvastatin, administered at 20 mg/kg for 4 consecutive days, might constitute an effective therapeutic neuroprotector against TBI-induced depression-like behavior via anti-neuroinflammation.[6] In this article, we further showed that simvastatin therapy, particularly at the 20 mg/kg dose, in the acute stage following TBI attenuates microglia and astrocyte activation, TNF-α expression, and neuronal apoptosis in rats with pre-existing hypercholesterol that were fed a high-fat diet.

The brain is very cholesterol-rich, and it contains approximately 20% of the whole body's cholesterol.[20] Demediuk et al.[21] reported that the total cholesterol level of the injured rat brain tissue was decreased significantly at 10 minutes, 4 hours, and 24 hours after TBI, which indicates the excess cholesterol may be due to injury-induced neuronal damage and resulting membrane debris. Kay et al.[4] demonstrated that, following TBI, patients show an increase in cholesterol in the CSF when

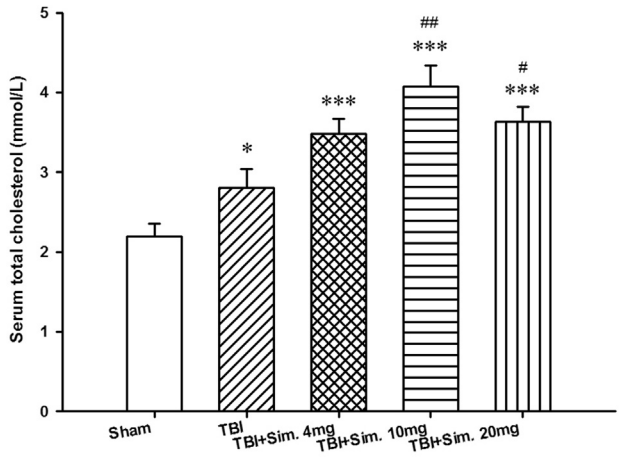

Figure 10. Effects of simvastatin (Sim.) treatment on serum total cholesterol concentration. Traumatic brain injury (TBI) significantly increased the total serum cholesterol concentration. However, total serum cholesterol remained high despite simvastatin treatment. $*P < 0.05$, $***P < 0.001$, $\#P < 0.05$, and $\#\#P < 0.01$ ($n = 6$ in each group).

sampled at admission. These authors proposed the increase in CSF cholesterol may result from the disturbance in the remodeling of lipoprotein particles and the recycling of cholesterol.

In current study, on the third day after TBI, even though we did not measure the cholesterol level in the central nervous system, we found that total serum cholesterol was significantly increased, and it further increased despite simvastatin treatment. It is possible that the high level of serum cholesterol may result from excretion of cholesterol from the injured brain because of the disrupted blood–brain barrier after TBI[22] and secondary injury following TBI. Since cholesterol is converted to bile salt in the liver, absorbed in the intestine, and carried by low-density lipoprotein,[22] we propose the brain–gut axis may play a role in the regulation of intestinal lipid and lipoprotein metabolism after TBI.[23] However, this possibility needs to be investigated in future work.

The neuroprotection of simvastatin in experimental TBI has been attributed to its anti-inflammatory effects on vascular endothelial inflammation,[10] the involvement of the TLR4/NF-kappaB pathway in the injured rat brain,[11] or the decrease in astromicroglia and TNF-α expression in the hippocampus.[6] In the current study, we contribute new information that simvastatin attenuated neuronal TNFR1 activation, microglial/astrocyte activation, TNF-α expression in activated microglia/astrocyte, and neuronal apoptosis in the peri-lesioned cortex in the high-fat diet–induced, high-cholesterol rats after TBI. One possible explanation for the decreased expression of the neuroprotector TNFR2 in the simvastatin-treated groups might be that fewer neurons are associated with lower TNFR2 expression after TBI.

The harmfulness of high cholesterol to the central nervous system has been reported, as it induces neuroinflammation or an immune response characterized by glial activation, proinflammatory cytokines, and cytokine expression in a ischemia brain injury model[24] and a low-density lipoprotein knock-out mouse model.[3] However, in a prospective study of critically ill surgical patients, Gordon et al.[25] found that patients with neurologic conditions had markedly less hypercholesterolemia in comparison with those without brain pathology. Krisanova et al.[26] reported that lowering cholesterol, which constitutes membrane proteins involved in synaptic transmission, may cause harmful consequences by decreasing glutamate uptake in nerve terminals. In the current study, we found that high levels of serum cholesterol in the statin groups significantly decreased neuroinflammation after TBI. Cholesterol is an essential structural component for the plasma membrane and myelin, maintaining neuronal physiology.[27] The optimal level of serum cholesterol in this specific condition needs to be clarified in the future.

Issues Worth Investigating in the Future

Several issues worth investigating in the future should be mentioned. First, in the current study, we focus on the effects of pre-hyperlipidemia hypercholesterol on TBI in the experimental rat. Whether these neurobehavioral and neuroinflammatory parameters were significantly different between rats fed with a high-fat diet and normal-diet rats after TBI is worth investigating.

Second, we chose only male subjects in light of the potential sex differences in cholesterol metabolism[28] and neuroprotective effects that occur following TBI, especially in the pre-existing hypercholesterol condition.

Third, in the current study, we only tested simvastatin effects on TBI using an insult of moderate severity (2.2 atm). Whether pre-existing hypercholesterol is harmful after mild (1.8 atm) and high severity (2.4 atm) TBI insults, and whether treatment with simvastatin has neuroprotective effects is worth clarifying.

Fourth, in the current study, given that acute pharmacologic interventions have shown short-term postinjury therapeutic effects, the long-term outcome of simvastatin on TBI-induced neuroinflammation could be investigated in follow-up studies.

Fifth, we did not measure the cholesterol level in brain tissue or CSF, which may accurately reflect the status of the time course of cholesterol metabolism in the rats with pre-existing hypercholesterol after TBI. Therefore, it is worth investigating the changes in the brain lipid profile over time following TBI in the hypercholesterol rats.

Finally, in our previous study, we demonstrated that pre-existing hyperlipidemia is an independent risk factor for the development of depression after TBI.[29] We also found that simvastatin therapy in the acute stage attenuates brain trauma-induced depression-like behavior in rats.[6] Therefore, we believe that, by using the hypercholesterol experimental animal model, we can conduct more in-depth investigations about the

associations among hypercholesterol, post-TBI depression, and statin treatment.

CONCLUSIONS

Our results show that the administration of simvastatin for 3 consecutive days in the acute stage following TBI counteracts neuroinflammation in the ischemic cortex and attenuates motor deficits induced by TBI in rats with pre-existing hypercholesterol, without decreasing the serum cholesterol levels. This effect might represent one mechanism responsible for the neuroprotective effect of simvastatin.

ACKNOWLEDGMENTS

The authors thank all of the researchers, especially Chiao-Ya Hu, who participated in this study.

REFERENCES

1. Adekunle AS, Adedeji AL, Oyewo EO, Adedosu OT, Omotoso AT. Hyperlipidemia induced by atherogenic diet enhanced oxidative stress in the kidney and inflammatory responses: an in-vivo study. *Asian J Nat Appl Sci*. 2013;2:82-93.
2. Diaz-Castro L, Cabello-Rangel H, Cuevas-Pineda GJ, Reza-Garduno H, Castaneda-Gonzalez CJ. Prevalence of the metabolic syndrome in a psychiatric hospital in Mexico. *Actas Esp Psiquiatr*. 2011;39:115-122.
3. Thirumangalakudi L, Prakasam A, Zhang R, et al. High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. *J Neurochem*. 2008;106:475-485.
4. Kay AD, Day SP, Kerr M, Nicol JA, Packard CJ, Caslake MJ. Remodeling of cerebrospinal fluid lipoprotein particles after human traumatic brain injury. *J Neurotrauma*. 2003;20:717-723.
5. Lim SW, Wang CC, Wang YH, Chio CC, Niu KC, Kuo JR. Microglial activation induced by traumatic brain injury is suppressed by postinjury treatment with hyperbaric oxygen therapy. *J Surg Res*. 2013;184:1076-1084.
6. Lim SW, Shiue YL, Liao JC, et al. Simvastatin therapy in the acute stage of traumatic brain injury attenuates brain trauma-induced depression-like behavior in rats by reducing neuroinflammation in the hippocampus. *Neurocrit Care*. 2017;26:122-132.
7. Dong Y, Fischer R, Naudé PJ, et al. Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. *Proc Natl Acad Sci U S A*. 2016;113:12304-12309.
8. Probert L. TNF and its receptors in the CNS: the essential, the desirable and the deleterious effects. *Neuroscience*. 2015;32:2-22.
9. Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. *Clin Pharmacokinet*. 1993;24:195-202.
10. Wang KW, Chen HJ, Lu K, et al. Simvastatin attenuates the cerebral vascular endothelial inflammatory response in a rat traumatic brain injury. *Ann Clin Lab Sci*. 2014;44:145-150.
11. 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.
12. Cucchiara B, Kasner SE. Use of statins in CNS disorders. *J Neurol Sci*. 2001;187:81-89.
13. Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. *Circ Res*. 2005;97:1232-1235.
14. Chuang TJ, Lin KC, Chio CC, Wang CC, Chang CP, Kuo JR. Effects of secretome obtained from normoxia-preconditioned human mesenchymal stem cells in traumatic brain injury rats. *J Trauma Acute Care Surg*. 2012;73:1161-11617.
15. Hallam TM, Floyd CL, Folkerts MM, et al. Comparison of behavioral deficits and acute neuronal degeneration in rat lateral fluid percussion and weight-drop brain injury models. *J Neurotrauma*. 2004;21:521-539.
16. Mullen RJ, Buck CR, Smith AM. Neu-N, a neuronal specific nuclear protein in vertebrates. *Development*. 1992;116:201-211.
17. Koshinaga M, Katayama Y, Fukushima M. Rapid and widespread microglial activation induced by traumatic brain injury in rat brain slices. *J Neurotrauma*. 2000;17:185-192.
18. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol*. 2004;19:117-125.
19. 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.
20. Bjorkhem I, Meaney S, Fogelman AM. Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol*. 2004;24:806-815.
21. Demediuk P, Faden AI, Romhanyi R, Vink R, McIntosh TK. Traumatic brain injury in the rat: effects on lipid metabolism, tissue magnesium, and water content. *J Neurotrauma*. 1988;5:105-119.
22. Zhang J, Liu Q. Cholesterol metabolism and homeostasis in the brain. *Protein Cell*. 2015;6:254-264.
23. Farr S, Taher J, Adeli K. Central nervous system regulation of intestinal lipid and lipoprotein metabolism. *Curr Opin Lipidol*. 2016;27:1-7.
24. Herz J, Hagen SI, Bergmüller E, et al. Exacerbation of ischemic brain injury in hypercholesterolemic mice is associated with pronounced changes in peripheral and cerebral immune responses. *Neurobiol Dis*. 2014;62:456-468.
25. Gordon BR, Parker TS, Levine DM, et al. Relationship of hypolipidemia to cytokine concentrations and outcomes in critically ill surgical patients. *Crit Care Med*. 2001;29:1563-1568.
26. Krisanova N, Sivko R, Kasatkina L, Borisova T. Neuroprotection by lowering cholesterol: a decrease in membrane cholesterol content reduces transporter-mediated glutamate release from brain nerve terminals. *Biochim Biophys Acta*. 2012;1822:1553-1561.
27. Goritz C, Mauch DH, Pfrieger FW. Multiple mechanisms mediate cholesterol-induced synaptogenesis in a CNS neuron. *Mol Cell Neurosci*. 2005;29:190-201.
28. Palmisano BT, Zhu L, Eckel RH, Stafford JM. Sex differences in lipid and lipoprotein metabolism. *Mol Metab*. 2018;15:45-55.
29. Wee HY, Ho CH, Liang FW, et al. Increased risk of new-onset depression in patients with traumatic brain injury and hyperlipidemia: the important role of statin medications. *J Clin Psychiatry*. 2016;77:505-511.

Conflict of interest statement: The authors declare that the article content was composed in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received 15 July 2019; accepted 30 August 2019

Citation: World Neurosurg. (2019) 132:e99-e108.
https://doi.org/10.1016/j.wneu.2019.08.250

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery***

Available online: ***www.sciencedirect.com***

1878-8750/$ - see front matter