

## Effect of atorvastatin on spatial memory, neuronal survival, and vascular density in female rats after traumatic brain injury

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**Object.** Atorvastatin administered after traumatic brain injury (TBI) induced by controlled cortical impact promotes functional improvement in male rats. Note, however, that parallel studies have not been performed in female rats. Therefore, the authors tested the effect of atorvastatin on TBI in female rats.

**Methods.** Atorvastatin (1 mg/kg/day) was orally administered for 7 consecutive days in female Wistar rats starting 1 day after TBI; control animals received saline. Modified neurological severity scores, the corner turn test, and the Morris water maze test were used to evaluate functional response to treatment. Rats were killed on Day 15 post-TBI, and brain tissue samples were processed for immunohistochemical staining. Atorvastatin administration after brain injury significantly promoted the restoration of spatial memory but did not reduce sensorimotor functional deficits. Treatment of TBI with atorvastatin increased neuronal survival in the CA3 region and the lesion boundary zone and prevented the loss of neuronal processes of damaged neurons in the hippocampal CA3 region but not in the lesion boundary zone on Day 15 after TBI. The protective effect of atorvastatin on the injured neurons perhaps is mediated by increasing the density of vessels in the lesion boundary zone and the hippocampus after TBI.

**Conclusions.** These data indicate that atorvastatin is beneficial in the treatment of TBI in female rats, although the effect may differ between sexes.

**KEY WORDS** • neuroplasticity • spatial memory • traumatic brain injury • atorvastatin • rat

EAch year, approximately 200 of 1 million individuals experience TBI. The population with TBI consists of approximately 25% female patients.<sup>25</sup> The effect of sex on TBI outcome is controversial. In a retrospective clinical study, Coimbra, et al.,<sup>4</sup> concluded that sex does not play a role in death following trauma or in the incidence of acute complications after any degree of closed-head injury. Note, however, that authors of other clinical studies assert that female persons may have a greater risk for worse long-term outcomes compared with male individuals.<sup>8,24</sup> In preclinical studies, Kupina and colleagues<sup>11</sup> found that peak protein degradation and neurodegeneration occurred within 3 days after TBI in male mice, whereas these processes did not occur until 14 days after TBI in female mice. Furthermore, 20% of male mice with TBI died within a short period immediately following injury, whereas all female mice with TBI survived despite having the same degree of damage. Female rats are reported to have smaller areas of damaged brain compared with those in both ovariectomized rats and male rats following parasagittal fluid-percussion brain inju-

ry, thus indicating a hormonally dependent neuroprotective effect.<sup>1</sup> Although the effect of sex on the pathophysiological mechanisms and degree of recovery following TBI are areas of intense investigation, what has not been studied is whether there is a sex-dependent response to therapeutic interventions for TBI, particularly neurorestorative therapy.

In an attempt to reduce neurological deficits and to rescue damaged neurons caused by TBI, we tested the effect of atorvastatin, a  $\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme A reductase inhibitor, in a controlled cortical impact model in the male rat.<sup>12</sup> Male rats treated with atorvastatin starting 1 day after TBI exhibit significant functional improvement in somatosensory response and a learning paradigm as well as concomitant induction of brain plasticity and a reduction in microvascular coagulation.<sup>14,15</sup> To our knowledge, however, there have been no studies focused on the response of female rats subjected to TBI and treated with atorvastatin. Note that differential sex effects of atorvastatin have been reported. For example, atorvastatin increases the anticoagulant effect of warfarin in postmenopausal women but not in men of the same age.<sup>19</sup> We therefore designed the present study to test the atorvastatin treatment effect on sensorimotor function and spatial learning as well as on induction of neuroplasticity in the injured brain of female rats subjected to TBI.

*Abbreviations used in this paper:* DG = dentate gyrus; MNSS = modified neurological severity score; PBS = phosphate-buffered saline; TBI = traumatic brain injury; VWF = von Willebrand factor.

## Materials and Methods

All experimental procedures were approved by the Care of Experimental Animals Committee of Henry Ford Hospital.

### Animal Model

A controlled cortical impact model of TBI in rats was used in the present study.<sup>7,18</sup> Female Wistar rats weighing 300 to 350 g each were anesthetized intraperitoneally with chloral hydrate (350 mg/kg body weight). Rectal temperature was maintained at 37°C by using a feedback-regulated water heating pad. A controlled cortical impact device was used to induce injury. Rats were placed in a stereotaxic frame. Two 10-mm-diameter craniotomies were performed adjacent to the central suture, midway between the lambda and bregma. The second craniotomy allowed for lateral movement of cortical tissue. The dura mater was kept intact over the cortex. Injury was induced by impacting the left cortex (ipsilateral cortex) with a pneumatic piston containing a 6-mm-diameter tip at a rate of 4 m/second and 2.5 mm of compression. Velocity was measured with a linear velocity displacement transducer. Brain injury in this model is characterized by cystic cavity formation in cortex, which causes asymmetric neurological deficits<sup>17</sup> and selective cell damage in hippocampal formation, causing spatial memory dysfunction.<sup>12</sup> Therefore, sensorimotor and spatial memory tests were used to evaluate functional response to injury and treatment after TBI.

### Sensorimotor Functional Tests

The measurement of sensorimotor function was performed using an MNSS<sup>2,13,23</sup> and a corner turn test.<sup>9,27</sup> These measures were conducted in all rats before injury and on Days 1, 5, 8, and 15 after TBI. The MNSS is a composite of motor (muscle status and abnormal movement), sensory (visual, tactile, and proprioceptive), beam balance, and reflex tests. Motor tests of the MNSS include seven items with a maximum score of 3 points, which mainly reflect the function of the motor representation area in the contralateral cortex. Damage to this area causes contralateral limb paralysis, leading to high scores on the MNSS motor tests. Sensory tests include two items with a maximum score of 2, reflecting a combination of visual, tactile, and deep sensations. A unilateral lesion in the sensory and motor representations of the forelimb in the somatosensory cortex can produce contralateral asymmetry.<sup>5,6,26</sup> The placing test, included as a sensory test of the MNSS, also reflects an aspect of motor function, because the corticospinal pathway mediates the execution of the placing reaction and lesions in this region produce an enduring forelimb-placing deficit.<sup>20</sup> Beam balance tests, part of the asymmetry test, contain seven items with a maximum score of 6, mainly reflecting hindlimb placing performance, which is controlled by the contralateral cortical representation of motor function. Damage to this area causes dragging of the contralateral hindlimb (the hindlimb is not placed on the beam), or the hindlimb is placed on the vertical surface of the beam to help support the animal's weight and to aid in maintaining balance, which reflects a high score on the beam balance tests. The last part of the MNSS includes the pinna, corneal and startle reflexes, and abnormal movements. In this model, injury in the left hemisphere cortex of rats causes sensory and motor functional deficits with elevated scores on motor, sensory, and beam balance tests in the early phase after injury (Day 1 postinjury).<sup>16</sup> Absent reflexes and abnormal movements are present in rats with severe injury.

The corner turn test was developed for measuring long-term functional recovery in the rat.<sup>9</sup> The test is sensitive to unilateral cortical injury because it reflects multiple asymmetries, including postural, vibrissae sensory, and fore- and hindlimb use asymmetries, which all combine to bias turning. An uninjured rat randomly turns left or right, whereas an injured rat preferentially turns toward the unimpaired ipsilateral (left) side. We recorded the number of right turns from 10 trials for each test and used the results for statistical analysis.<sup>9</sup> Recovery in asymmetry deficiency as reflected by the beam balance test and the corner turn test has been reported in unilateral brain injury such as brain trauma,<sup>21</sup> brain hemorrhage,<sup>9</sup> and stroke.<sup>27</sup> These tests are suitable for neurological functional evaluation after unilateral brain injury.

### Spatial Memory Test Procedures

Our spatial memory testing procedure is a modification of the Morris water maze test, as described previously.<sup>5,6,12,26</sup> Data collection was automated using the HVS Image 2020 Plus Tracking System (US HVS Image, San Diego, CA). For the training trials, each animal performed one trial per day for a 5-day session, with a 3-day break between the final training session and brain injury. The rats were then tested on Days 4, 8, and 15 after TBI or surgery. At the start of a trial, the rat was randomly placed at one of four fixed starting points, randomly facing either toward the wall or inwardly (designated north, south, east, and west), and was allowed to swim for 90 seconds or until it found the platform. The platform was located in a randomly changing position within the northeast quadrant throughout the test period (for example, sometimes equidistant from the center and edge of the pool, against the wall, near the center of the pool, or at the edges of the northeast quadrant). If the animal was unable to find the platform within 90 seconds, the experiment was terminated and a maximal score of 90 seconds was assigned. The percentage of time traveled within the northeast (correct) quadrant was calculated relative to the total amount of time spent swimming before reaching the platform.

### Experimental Groups

Ten female Wistar rats were randomly divided into two groups (five animals/group). Five rats were subjected to TBI followed by the oral administration of atorvastatin (1 mg/kg/day) 1 day after TBI for 7 consecutive days. An additional five rats subjected to TBI were treated with oral saline at the same dose and for the same period. The atorvastatin dose was selected based on prior work on stroke and TBI.<sup>12</sup> All rats were killed 15 days after TBI.

### Tissue Preparation

Fifteen days after TBI, the rats were anesthetized intraperitoneally with ketamine and xylazine and were perfused transcardially with saline solution containing heparin, followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Their brains were removed, postfixed in 10% formalin for 1 to 2 days at room temperature, and then processed for paraffin sectioning. A series of 6- $\mu$ m-thick tissue sections were cut using a microtome through each of seven standard blocks. A section from every block was stained with H & E for the calculation of lesion volume.

### Immunofluorescence Studies

After dehydration, tissue sections were boiled in 1% citric acid buffer (pH 6) in a microwave oven for 10 minutes, cooled to room temperature, and incubated in 1% saponin for 3 hours. Subsequently, the sections were incubated in 1% bovine serum albumin to block the nonspecific signals. Using the same buffer solution, the sections were incubated overnight at 4°C in primary antibody (monoclonal mouse anti-MAP2, dilution 1:400; Chemicon, Temecula, CA), followed by 2 hours at room temperature in corresponding fluorochrome-conjugated secondary antibody (anti-mouse fluorescein isothiocyanate; Jackson ImmunoResearch, West Grove, PA). Each of the aforementioned steps was followed by four 5-minute rinses in PBS. The sections were counterstained with propidium iodide for the identification of nuclei. Tissue sections were mounted on slides with ProLong antifade medium (Molecular Probes, Eugene, OR). Sections were observed with the aid of a fluorescent microscope.

### Immunoperoxidase Staining

To identify the vascular structure, brain tissue sections, after being deparaffinized, were incubated in 2% bovine serum albumin/PBS at room temperature for 30 minutes and subsequently were treated with mouse anti-VWF antibody (Dako Cytomation, Carpinteria, CA) diluted to 1:200 in PBS at 4°C overnight. Following sequential incubation with biotin-conjugated anti-mouse immunoglobulin G (dilution 1:100; Dakopatts, Carpinteria, CA), the sections were treated with an avidin-biotin-peroxidase system (ABC kit; Vector Laboratories, Inc., Burlingame, CA). Diaminobenzidine was then used as a sensitive chromogen for light microscopy.

## Effect of atorvastatin in female rats post-traumatic brain injury

TABLE 1  
*Neurological functional response after atorvastatin treatment in rats subjected to TBI\**

Test	Preinjury	Postinjury Day		
		1	8	15
MNSS				
control	0	14 ± 1.3	6.0 ± 1.4	3.4 ± 1.4
atorvastatin-treated	0	13 ± 0.9	6.2 ± 0.8	3.8 ± 1.3
corner turn test				
control	4.8 ± 0.5	2.0 ± 0.5	3.0 ± 1.0	3.8 ± 0.8
atorvastatin-treated	5.0 ± 0.7	2.2 ± 0.7	2.4 ± 1.5	3.8 ± 1.6

\* Values are presented as the means ± standard deviations.

### Cell Counting

To evaluate whether orally administered atorvastatin reduces neuronal damage after TBI, cell counts were performed by observers blinded to the individual treatment status of the animals. The MAP2-positive cells were defined as surviving neurons and counted in the CA3 region of the hippocampal formation and the boundary zone of the injured cortex. Counting of neurons with axons and dendrites was also performed in these regions in the tissues from the two groups, and the percentage of neurons with an axon and dendrites was used as a parameter to evaluate the effect of the atorvastatin treatment. Five tissue sections cut at 50- $\mu$ m intervals through the dorsal DG were analyzed with the aid of a fluorescent microscope  $\times$  200 (at the interaural 5.2-mm levels). Using an image analyzer (MCID; St. Catharines, ON, Canada), the numbers of MAP2-positive cells were counted as surviving neurons per square millimeter in the CA3 region of the ipsilateral hippocampus.

### Boundary Zone of the Injured Cortex

The MAP2-positive cells in the boundary zone of the injury were counted as the surviving neurons per square millimeter on the same sections as those used for the hippocampus. The boundary zone was defined as the area surrounding the lesion cavity, which morphologically differs from surrounding intact brain tissue. Surviving neuronal cells were calculated as a density (cell number per square millimeter) in the lesion boundary zone and as the number of MAP2-positive cells per millimeter in the hippocampal CA3 region.

### Volumetric Analysis

To estimate the lesion volume of the cortex, tissue sections were stained with H & E and were analyzed using a 10 $\times$  objective and a computer imaging analysis system.<sup>28</sup> Lesion volume was calculated by measuring the area of the lesion, including the lesion cavity and boundary zone, from each section and multiplying the lesion area by the section thickness and the sampling intervals.

### Measurement of Vascular Density

Five sections with 50- $\mu$ m intervals through the dorsal DG were stained for VWF, and the images were digitized using a light microscope  $\times$  400 (at the interaural 5.2-mm levels). The VWF-positive vessels were counted in the boundary zone of the lesion and the CA3 region of the hippocampus by using the image analyzer system (MCID). The vascular density in both regions was determined by dividing the number of immunoreactive vessels by the corresponding area.<sup>28</sup>

### Statistical Analysis

All data are presented as the means ± standard deviation. Data were analyzed using an analysis of variance for repeated measures of functional test data.<sup>17</sup> A paired t-test was used to consider the difference in cell counts as well as in vascular density in the ipsilateral

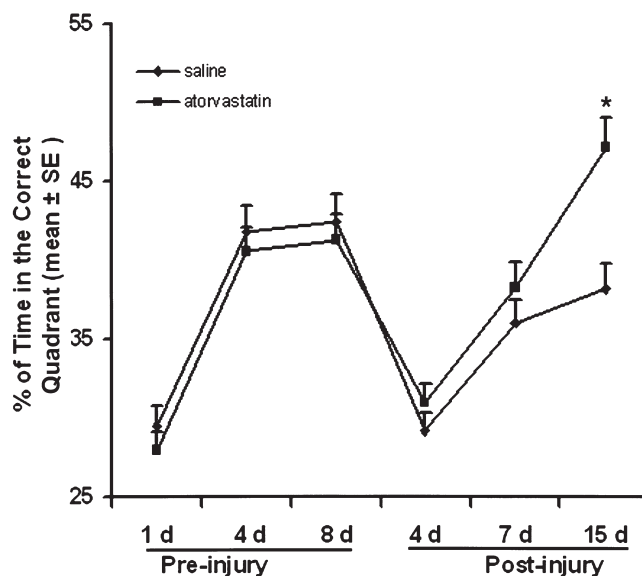


FIG. 1. Graph showing the effect of atorvastatin on the spatial memory in female rats after TBI, measured by the Morris water maze test. Preinjury training of female rats significantly augmented the time spent in the correct quadrant, which was measured 4 and 8 days following initial training. At 4 days post-TBI, the time spent in the correct quadrant significantly decreased, and atorvastatin treatment significantly increased the restoration of spatial memory on Day 15 after TBI when compared with that in the saline-treated group (\* $p < 0.05$ ). These data demonstrate that the Morris water maze test is a sensitive test by which the enhancement, deficits, and restoration of the spatial memory function after TBI can be measured. Atorvastatin significantly promotes improvement of spatial memory after TBI in female Wistar rats. SE = standard error.

hemisphere between the atorvastatin-treated group and the control group. All measures were analyzed by observers blinded to individual treatments.

## Results

### Neurological and Sensorimotor Functional Responses

Injury in the left hemisphere cortex in rats caused neurological functional deficits as measured using the MNSS. These rats presented with high scores on motor, sensory, and beam balance tests on Day 1 postinjury (Table 1). Absent reflexes and abnormal movements were evident in rats with severe injury. By Day 15 postinjury, residual deficit scores were associated mainly with the beam balance tests and the sensory (placing) test of the MNSS. The MNSS scores for the atorvastatin-treated group ( $3.4 \pm 1.1$ ) were not significantly decreased on Day 15 after TBI when compared with those in the saline-treated group ( $3.8 \pm 1.3$ ;  $p > 0.05$ ). There was also no significant difference on the corner turn test between the atorvastatin- and saline-treated animals at any time point. These data demonstrate that atorvastatin does not significantly reduce asymmetry deficiencies caused by TBI in female Wistar rats.

### Spatial Memory Test

On the 1st day of training, the mean percent of time spent

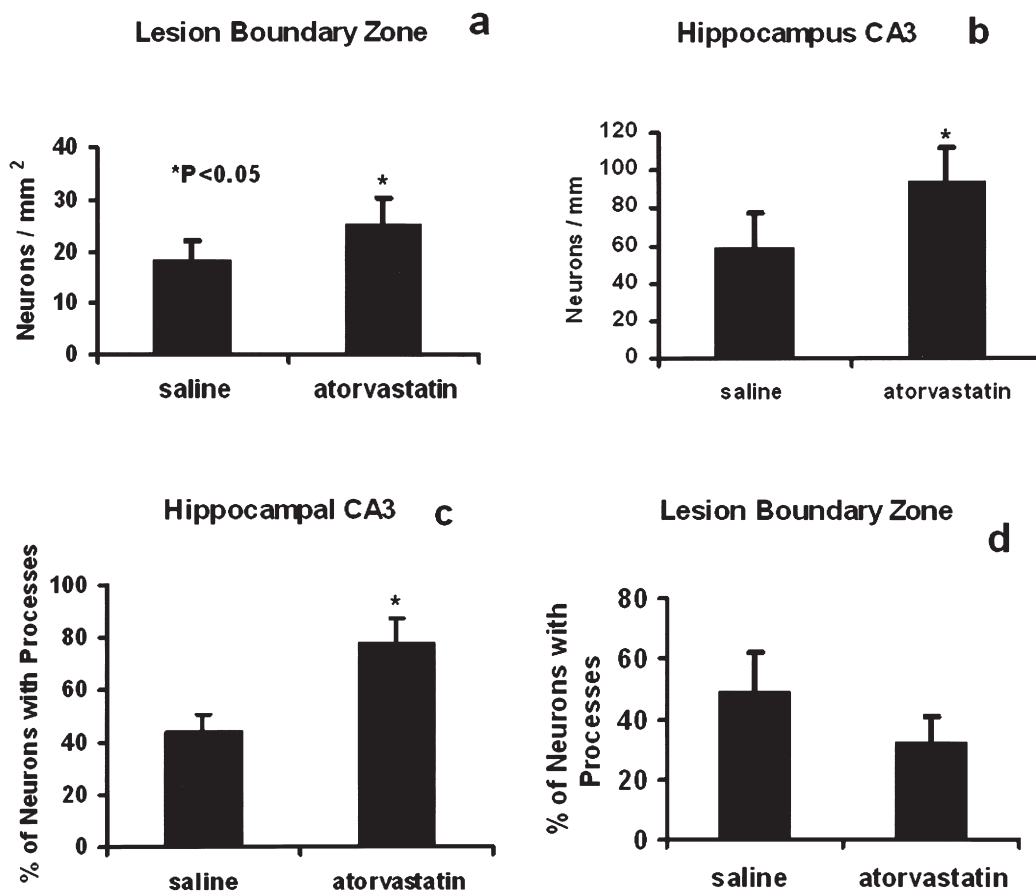


FIG. 2. Bar graphs showing atorvastatin-augmented numbers of surviving neurons in the lesion boundary zone (a) and the hippocampal CA3 region (b) and percentages of neurons with processes in the hippocampal CA3 region (c) but not in the lesion boundary zone (d).

in the correct quadrant (containing the platform) was approximately 27% and persistently increased during the subsequent training days, reaching approximately 40% after five daily training sessions (Fig. 1). The rats subjected to TBI spent a significantly diminished percentage of time in the correct quadrant 4 days after TBI. These data demonstrate that brain damage in this model causes significant dysfunction of spatial memory in female rats, results that can indicate a target for the treatment of the injury.

The percentage of time spent in the correct quadrant was significantly greater in the atorvastatin-treated group on Day 15 post-TBI ( $47.1 \pm 8.8\%$ ) compared with that in the saline-treated group ( $38 \pm 2.29\%$ ;  $p < 0.05$ ). These data demonstrate that atorvastatin administration enhances the restoration of spatial memory function damaged in TBI in female Wistar rats.

#### Lesion Volume

Using the image analyzer system, the lesion volume, including both the cavity and the lesion boundary zone in the cortex, was calculated in both groups. There was no significant difference in lesion volume between the atorvastatin- and saline-treated groups ( $9.38 \pm 3.05\%$  compared with  $10.66 \pm 5.95\%$ , respectively;  $p > 0.05$ ). These data demonstrate that atorvastatin does not significantly reduce lesion volume in female rats after TBI.

#### Neuron Survival in the Boundary Zone and the CA3 Region of the Hippocampus

Surviving neurons in the lesion boundary zone and the hippocampal CA3 region were counted on the MAP2-stained slides. Compared with those in the contralateral hemisphere, surviving neurons in the lesion boundary zone after TBI lose pyramidal cell features, such as a long axon, and many processes. The density of the neuronal cells in the lesion boundary zone of the atorvastatin-treated group ( $25.7 \pm 5.2/\text{mm}^2$ ) was significantly greater than that in the saline-treated group ( $16.5 \pm 3.4/\text{mm}^2$ ;  $p < 0.05$ ; Fig. 2a). In the hippocampal CA3 region, atorvastatin treatment significantly enhanced survival of pyramidal cells ( $94.6 \pm 17.2/\text{mm}$ ) when compared with saline treatment ( $58.9 \pm 18.8/\text{mm}$ ;  $p < 0.05$ ; Fig. 2b), indicating that atorvastatin can play a role in protecting damaged neurons in the lesion boundary zone and hippocampal areas from death induced by TBI.

Morphologically, neurons in the pyramidal cell layer of the CA3 region in the contralateral hippocampus have a polygonal large body with a long axon projecting to the stratum lucidum (Fig. 3a1 and a2). After TBI, pyramidal cells in the CA3 region lose these normal features and there is significant loss of axons and dendrites (Fig. 3b1 and b2). The percentage of surviving neurons with an axon and dendrites in the CA3 region ( $78 \pm 9\%$ ) following atorvastatin

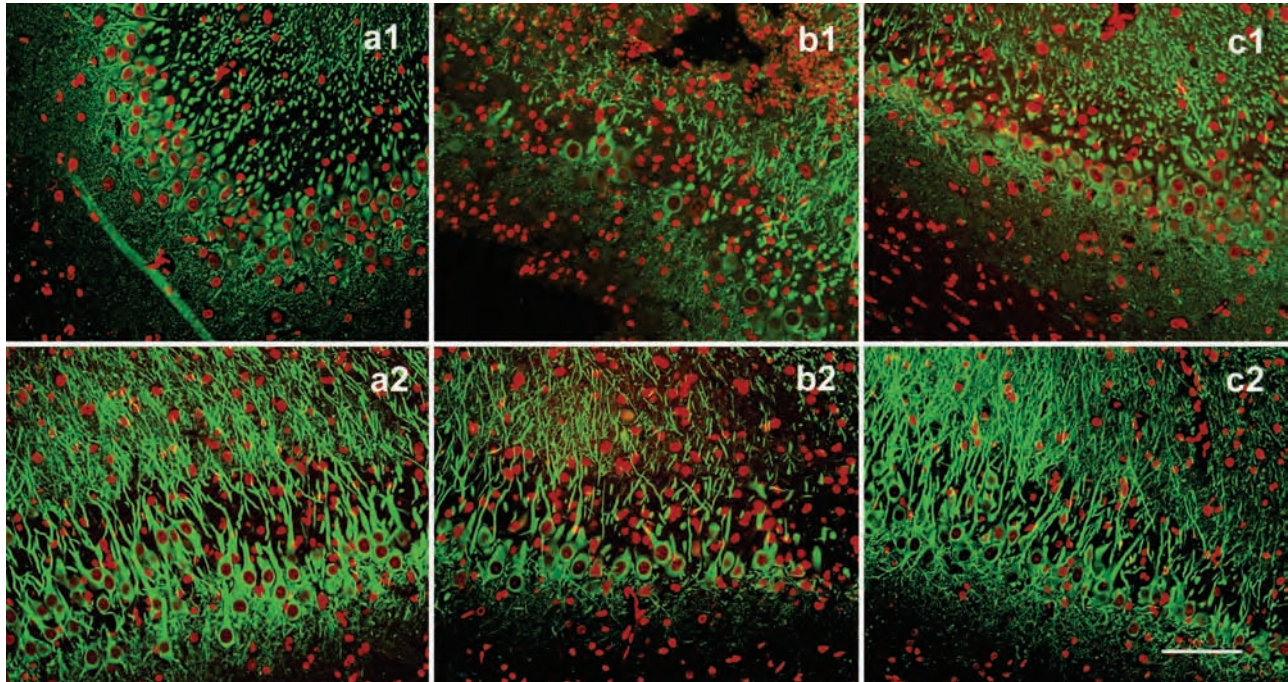


FIG. 3. Photomicrographs showing MAP2 staining (green) and counterstaining with propidium iodide (red). The CA3 region close to the DG (a1) and the CA3 region adjacent to the CA2 region (a2) reveal neurons marked by MAP2 in the contralateral hippocampal CA3 region. b1 and b2: Decreased numbers of neurons are present in the CA3 region with saline treatment after TBI. c1 and c2: Atorvastatin increases neuronal survival in the CA3 region after TBI. Pyramidal cells with long axons and many dendrites are present in the contralateral CA3 regions (a2) and a reduction of pyramidal cells and their processes appears after TBI (b2). Significantly, more MAP2-positive cells are found in the CA3 region after atorvastatin treatment (c1 and c2) when compared with the saline-treated (b1 and b2) group, but less than in the contralateral CA3 region (a1 and a2). Bar = 50  $\mu$ m.

treatment is significantly increased compared with that following saline treatment ( $44 \pm 7\%$ ;  $p < 0.05$ ; Fig. 2c). In the atorvastatin-treated group, many surviving neurons exhibit features of normal neurons, such as the long axon and numerous dendrites (Fig. 3c1 and c2). No significant difference occurred in the percentage of surviving neurons with processes in the lesion boundary zone between the atorvastatin- and saline-treated groups ( $39 \pm 13\%$  compared with  $32 \pm 9\%$ , respectively;  $p > 0.05$ ; Fig. 2d). These data demonstrate that atorvastatin reduces the loss of the neurons and their processes in the CA3 region but not in the lesion boundary zone in female Wistar rats subjected to TBI.

*Vascular Changes After TBI*

The numbers of vessels were measured in the lesion boundary zone of the injured cortex and the CA3 region of the hippocampus by counting VWF-positive vessels per square millimeter, regardless of the vessel size. Vessel density was significantly greater in the atorvastatin-treated group than in the saline-treated group in the lesion boundary zone ( $34 \pm 5/\text{mm}^2$  compared with  $18 \pm 1.6/\text{mm}^2$ ,  $p < 0.05$ ) and in the hippocampal CA3 region ( $19 \pm 3/\text{mm}^2$  compared with  $7 \pm 2/\text{mm}^2$ ,  $p < 0.05$ ; Fig. 4). These data demonstrate that atorvastatin enhances vascular density in the injured brain in female rats after TBI.

**Discussion**

Our main findings in this study regarding atorvastatin

treatment of TBI in the female rat are as follows. Atorvastatin treatment significantly improves spatial memory but not sensorimotor functional outcome, augments the number of surviving neurons in the lesion boundary zone and hippocampal CA3 region, enhances the percentage of the neurons with processes in the hippocampal CA3 region, and increases vascular density in these two regions.

In the controlled cortical impact model of TBI, the impact selectively damages the pyramidal hippocampal CA3 region and causes contusion of the cortex in the ipsilateral hemisphere. These pathological changes contribute to deficits in neurological function, including sensorimotor

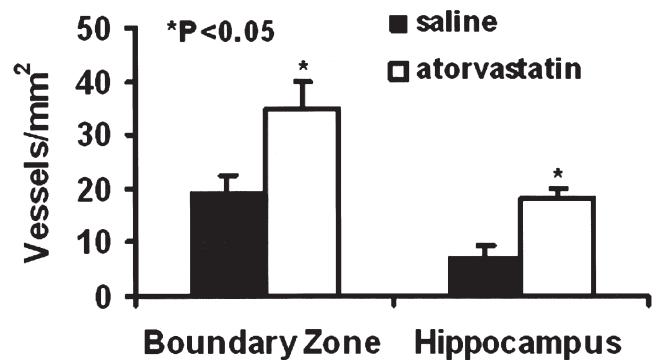


FIG. 4. Bar graphs showing significant increases in the density of vessels in the lesion boundary zone and the hippocampus.

and memory function.<sup>15,17,19</sup> The neurological function tests (MNSS, corner turn test, and Morris water maze test) in this study have been used to evaluate the functional responses and the effect of drugs on the functional recovery in rats subjected to TBI.<sup>13</sup> Our data demonstrate that damage to the cortex induces significant asymmetric functional responses in female rats, as reflected by the MNSS and results of the corner turn test, and a deficiency in spatial memory, as measured by the Morris water maze test. Treatment with atorvastatin for 1 week significantly promotes recovery of the spatial memory function on Day 15 after TBI. Note, however, that no significant difference was measured on the MNSS and the corner turn test between the atorvastatin- and saline-treated groups at this time point. In contrast, results of our previous study in male rats showed that atorvastatin significantly improves sensorimotor and spatial memory functions in male rats 15 days post-TBI.<sup>15</sup> Therefore, based on these data, one can infer that atorvastatin treatment causes different functional responses to the same treatment in male and female rats despite similar brain damage. The reason for the sex effect on atorvastatin treatment of TBI is unknown. The present study was not designed to compare directly the effect of sex on responses to treatment with atorvastatin. Nevertheless, we noted that the volume of the cerebral infarct in male rats was 23.4%<sup>13</sup> compared with 10.7% in the female rats reported on here. Thus, we may speculate that the intrinsic neuroprotection in female rats, which is characterized by a smaller lesion, may alter the response pattern of the statin therapy. Note, however, that the functional deficits, as measured using the MNSS (Score 12) and the corner turn test (Score 1) 1 day after TBI, were similar to values in the present study in the female rat (MNSS 13 and corner turn test Score 2). Thus, we cannot attribute the differences in the response to statin treatment to differences in somatosensory deficits, although the lesion volumes appear to be different. Additional studies are required to compare directly within a single study the sex effects on statin treatment of TBI.

Histological data revealed that atorvastatin treatment significantly enhances the number of surviving neurons in the lesion boundary zone and the hippocampal CA3 region but does not reduce lesion volume on Day 15 after TBI. These data are consistent with the findings from the study in male rats with TBI, indicating that the effects of atorvastatin in female rats with TBI can also be mediated by the modulation of cellular constituents in the lesion boundary zone and the hippocampal CA3 region post-TBI.<sup>15</sup> Increased numbers of surviving neurons in the hippocampal CA3 region have been reported to improve the spatial learning outcome in rats and mice post-TBI<sup>10</sup>—results that can be attributed to a more intact neural circuit after atorvastatin treatment compared with that in the control rat. Nevertheless, no significant improvement in sensorimotor function was measured following atorvastatin treatment in female rats compared with saline treatment. Although the number of surviving neurons increased in the lesion boundary zone, the percentage of neurons with processes did not. This result perhaps contributed to the lack of sensorimotor function differences between atorvastatin- and saline-treated groups.

Our current and previous data indicate that atorvastatin treatment reduces neuron loss and the loss of neuronal processes in the lesion boundary zone and the hippocampal CA3 region post-TBI in both female and male rats.<sup>12</sup> The

mechanisms underlying this protection perhaps are mediated by a reduction in secondary injury post-TBI. Atorvastatin reduces delayed thrombosis, maintains the integrity of the microvasculature, and induces angiogenesis, all of which enhance blood perfusion in the areas of interest.<sup>14,15</sup> As demonstrated in this study, atorvastatin increases vascular density in the lesion boundary zone and the hippocampal CA3 region. Atorvastatin also reduces damage caused by the intracerebral hemorrhage to surrounding neurons.<sup>22</sup> Endogenous neuroplasticity may also be enhanced by the atorvastatin treatment via increasing neurogenesis and synaptogenesis following brain injury.<sup>3,12</sup>

## Conclusions

Atorvastatin improves spatial memory but not sensorimotor function in female rats after TBI. This beneficial effect of atorvastatin perhaps is mediated by the reduction in neuronal death and the loss of neuronal processes as well as enhanced vascular density in the lesion boundary zone and the hippocampal CA3 regions after TBI.

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