Strong Reduction of Low-Density Lipoprotein Receptor/Apolipoprotein E Expressions by Telmisartan in Cerebral Cortex and Hippocampus of Stroke Resistant Spontaneously Hypertensive Rats

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Background: Telmisartan is a unique angiotensin II type 1 receptor blocker with a partial peroxisome proliferator-activated receptor–γ (PPARγ) agonistic property to exert not only antihypertensive effect but also antimetabolic syndrome effect. *Methods:* We examined the long-term effect of telmisartan on cholesterol transport-related proteins (low-density lipoprotein receptor [LDL-R]/apolipoprotein E [ApoE]) and microtubule-associated proteins 2 (MAP2) in the brains of stroke resistant spontaneously hypertensive rats (SHR-SRs), which were divided into 3 experiment groups including vehicle group (SHR/Ve), low-dose telmisartan group (SHR/Low, .3 mg/kg/day), and high-dose telmisartan group (SHR/High, 3 mg/kg/day). *Results:* The numbers of LDL-R– and immuno-ApoE–positive neurons increased in both cerebral cortex and hippocampus of SHR/Ve throughout 6, 12, and 18 months of age, compared with age-matched normotensive Wistar rats. On the other hand, telmisartan significantly reduced the numbers of LDL-R– and ApoE immuno-positive neurons in both cerebral cortex and hippocampus, with similar effectiveness in the SHR/Low group without blood pressure (BP) lowering to BP lowering (SHR/High). The decrease of MAP2-positive neuron in SHR/Ve was recovered by telmisartan in both cerebral cortex and hippocampus. *Conclusions:* These findings suggest that a long-term treatment with telmisartan directly improved neuronal lipid metabolism in the cerebral cortex and hippocampus of SHR-SR, mainly improving LDL-R and ApoE metabolism (SHR/Low) with a small additive benefit by BP lowering (SHR/High), which could provide a preventative approach in patients with hypertension at risk of Alzheimer disease. **Key Words:** Alzheimer's disease—spontaneously hypertensive rat—telmisartan—ApoE—LDL-R.

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Introduction

The worldwide aging is associated with an increased risk of Alzheimer disease (AD),[1,2] which is characterized neuropathologically by abnormal accumulation of senile plaques and neurofibrillary tangles throughout the cerebrocortical and limbic regions.[3] A part of the pathomechanism underlying amyloid-β peptide accumulation may be related to lipid peroxidation.[4-6] In fact, previous studies showed that apolipoprotein E (ApoE) and low-density lipoprotein receptor (LDL-R) participate in cholesterol transport in the brain, and ApoE plays a major role in modulating amyloid-β production and clearance in cooperation with LDL-R.[7-9] However, there has been no disease-modifying therapy for AD.[10-12]

A previous clinical study demonstrated that antihypertensive drugs via the renin–angiotensin system have a potential in preventing and delaying cognitive decline in hypertensive patients.[13] Telmisartan is a unique angiotensin II type 1 receptor blocker with a partial peroxisome proliferator-activated receptor–γ (PPARγ) agonistic property to exert not only antihypertensive effect but also antimetabolic syndrome effect.[14] However, little was known whether if telmisartan has long-term protective effects on lipid metabolism in brain relating to metabolic syndrome and dementia.

In the present study, therefore, we examined the long-term effect of telmisartan on LDL-R/ApoE expressions in brains of stroke resistant spontaneously hypertensive rats (SHR-SRs) as a hypertensive and vascular dementia model of rats.[15]

Materials and Methods

Animals and Drug Preparation

Seven-week-old male Wistar rats and SHR-SRs were provided from Disease Model Cooperative Research Association (Kyoto, Japan) and placed on a basal diet.

When the rats reached 3 months of age, the previously mentioned Wistar rats (n = 20) were started on a daily dose of .5% methylcellulose (MC) in 0.1 mL water by oral gavage as normotensive control group for the subsequent 3, 9, and 15 months until being killed by oral gavage, and the previously mentioned SHR-SRs (n = 54) were divided into the following 3 groups as treatment groups, that is, SHR-SR vehicle group (SHR/Ve, n = 17), SHR-SR low-dose telmisartan group in which the blood pressure (BP) did not fall significantly (SHR/Low, n = 19), and SHR-SR high-dose telmisartan group in which the BP fell by 30 mm Hg or more (SHR/High, n = 18), receiving daily oral doses of .5% MC only (SHR-SR/Ve), .5% MC plus low-dose telmisartan (0.3 mg/kg/day), or .5% MC plus high-dose telmisartan (3 mg/kg/day) for the subsequent 3, 9, and 15 months until being killed by oral gavage, respectively. The dose of telmisartan was determined as previously described.[16,17] Telmisartan was provided by Boehringer Ingelheim (Ingelheim am Rhein, Germany) and was given to the 2 rat groups as a suspension with .5% MC in .1 mL water every day. BP data in each experimental group was previously reported.[18]

At 6, 12, or 18 months of age, the rats were transcardially perfused with 5 U/mL chilled heparinized saline followed by 4% paraformaldehyde in phosphate buffer under deep anesthesia with pentobarbital (20 mg/250 g rat). After decapitation, their brains were removed, and the brain weights were measured. All experimental procedures were approved by the Animal Committee of the Graduate School of Medicine and Dentistry, Okayama University.

Immunohistochemistry

After the removal, the brains were immersed and fixed in 4% paraformaldehyde with .1 M phosphate buffer (pH 7.6) for 8 hours, embedded in paraffin, and 5 μm-thick sections were prepared for subsequent immunostaining. For LDL-R, ApoE, and microtubule-associated protein-2 (MAP2) immunostainings, the brain sections were pretreated by heating them 3 times in a 500-W microwave for 5 minutes in 10 mM (pH 6.0) citric acid buffer. These pretreated sections were then immersed in .5% periodic acid to block intrinsic peroxidase and treated with 5% normal horse serum in 50 mM phosphate-buffered saline (pH 7.4) containing .05% Tween 20 to block any nonspecific antibody response and were finally incubated overnight with each primary antibody. The following primary antibodies were used in this study: rabbit anti-LDL-R antibody (1:100; Epitomics, Burlingame, CA), goat anti-ApoE antibody (1:100; Millipore, Billerica, MA), and mouse anti-MAP2 antibody (1:200; Millipore). Biotinylated antibodies for LDL-R, ApoE, and MAP2 staining were used as the secondary antibodies, and specific labeling was visualized by a Vectastain Elite ABC kit (Vector, Burlingame, CA). To guarantee specific staining primary antibodies, brain sections were also stained without primary antibodies.

Detection and Analyses

The previously mentioned stained sections were digitized with a digital microscope camera (Olympus BX-51; Olympus Optical Co, Japan). Then the numbers of LDL-R–positive neuron, ApoE-positive neuron, and MAP2-positive neuron per each 1 mm^2 cerebral cortex in hemispheric coronal sections were counted at 3 levels (frontal cortex, basal ganglia, and posterior hippocampus). Three serial sections were used for each level, and all cell counts in the cerebral cortex per animal were determined and added. Furthermore, LDL-R–, ApoE-, and MAP2-positive neurons were quantified by counting stained cells in the CA1 and CA3 regions of the bilateral hippocampus and expressed as the percentage of LDL-R–, ApoE-, and MAP2-positive neurons over the total number of neurons in those regions.

Data are expressed as mean ± standard deviation. Statistical analyses were performed using analysis of variance with repeated measures (multiple comparisons). Planned comparisons were used for Tukey–Kramer post hoc analysis. $P < .05$ was considered significant. All statistical analyzes were performed with Statcel statistical package (Statcel 2; OMS Inc, Tokorozawa, Japan).

Recently, we have reported that telmisartan reduces progressive Alzheimer pathology with inflammatory responses in aged SHR-SRs.[18] And the present study on LDL-R, ApoE, and MAP2 focused on lipid-related aspect of comprehensive study on clinical, oxidative stress, inflammatory, and metabolic syndrome aspects that are under preparation for future submission.

Results

Immunohistochemical Analyses in the Cerebral Cortex

LDL-R Staining in Cerebral Cortex

In Wistar rats, LDL-R was only weakly in cerebrocortical neurons at 6-18 months of age (Fig 1, A, a, e, and i). Quantitative analysis showed that the mean numbers of LDL-R–positive neurons in 1 mm^2 of cerebral cortex were 38.5 ± 23.9 at 6 months, 44.1 ± 10.9 at 12 months, and 48.8 ± 9.4 at 18 months, respectively (Fig 1, B, open bars).

On the other hand, LDL-R was much clearly detectable in the dendrites (Fig 1, A, arrowheads) and cytoplasm (Fig 1, A, arrows) of cerebrocortical neurons throughout 6-18 months of age in SHR/Ve group. LDL-R–positive neurons were already evident at 6 months (124.4 ± 36.0/mm^2, $^{**}P < .01$ vs. Wistar group), but the number of cerebral cortex decreased with age (67.4 ± 26.8 at 12 months and 61.0 ± 12.9 at 18 months; Fig 1, A, b, f, and j and Fig 1, B).

In the 2 telmisartan-treated groups, the numbers of LDL-R–positive neurons of cerebral cortex were similar to Wistar rats with 49.9 ± 15.9 at 6 months (/mm^2, $\#\#P < .01$ vs. SHR/Ve group), 50.8 ± 27.6 at 12 months, and 49.1 ± 16.6 at 18 months in the SHR/Low group. In the SHR/High group, the numbers of LDL-R–positive neurons of cerebral cortex were 45.5 ± 9.3 at 6 months (/mm^2, $\#\#P < .01$ vs. SHR/Ve group), 47.5 ± 13.2 at 12 months, and 49.4 ± 18.2 at 18 months (Fig 1, B).

ApoE Staining in the Cerebral Cortex

In Wistar rats, ApoE-positive neurons were scarcely detectable in cerebral cortex at 6-18 months of age (Fig 2, A, a, e, and i). The mean numbers of ApoE-positive neurons of cerebral cortex were 16.1 ± 11.5 at 6 months, 41.9 ± 14.2 at 12 months, and 44.6 ± 18.2 at 18 months (Fig 2, B, open bars).

On the other hand, ApoE was clearly labeled in the cytoplasm of cerebrocortical neurons at 6 months in the SHR/Ve group, which gradually became stronger at 12 and 18 months (Fig 2, A, b, f, and j, arrowheads). The numbers of ApoE-positive neurons in cerebral cortex were 87.7 ± 25.4 at 6 months (/mm^2, $^{**}P < .01$ vs. Wistar group at 6 months), 92.0 ± 35.8 at 12 months ($^{**}P < .01$ vs. Wistar group at 12 months), and 132.9 ± 50.9 at 18 months ($^{**}P < .01$ vs. Wistar group at 18 months; Fig 2, B).

In the 2 telmisartan-treated groups, ApoE expression was decreased in cerebrocortical neurons at 6 months (Fig 2, A, c and d, arrowheads), which showed only a slight increase in the cytoplasm at 12 and 18 months (Fig 2, A, g, h, k, and l). The number of ApoE-positive neurons of cerebral cortex in the SHR/Low group was 45.7 ± 11.7 at 6 months (/mm^2, $\#\#P < .01$ vs. SHR/Ve group at 6 months), 60.2 ± 16.8 at 12 months, and 63.3 ± 13.7 at 18 months ($\#\#P < .01$ vs. SHR/Ve group at 18 months), and those in the SHR/High group was 39.8 ± 19.6 at 6 months ($\#\#P < .01$ vs. SHR/Ve group at 6 months), 43.7 ± 16.8 at 12 months ($\#\#P < .01$ vs. SHR/Ve group at 12 months), and 45.5 ± 20.7 at 18 months ($\#\#P < .01$ vs. SHR/Ve group at 18 months; Fig 2, B).

MAP2 Staining in the Cerebral Cortex

MAP2 was detectable in the dendrites and spines of cerebrocortical neurons throughout 6-18 months of age (Fig 3, A). Compared with Wistar group, the intensity of staining was slightly weaker in the SHR/Ve group at 6 months and in the 2 groups of telmisartan-treated SHR-SR.

Quantitative analysis of pixel intensity for MAP2 showed that the pixel intensities relative to that in the 6-month Wistar group were .65 ± .22 at 6 months in the SHR/Ve group, 1.15 ± .05 at 6 months in the SHR/Low group, and .95 ± .19 at 6 months in the SHR/High group (Fig 3, A, a-d and Fig 3, B). At 12 months of age, Wistar, SHR/Ve, and SHR/Low groups showed approximate conservation of MAP2 staining; however, the staining intensity became stronger in the SHR/High group than in Wistar group. The pixel intensities relative to that in the 6-month Wistar group were 1.10 ± .11 at 12 months in the SHR/Ve group, 1.15 ± .05 at 12 months in the SHR/Low group, and 1.09 ± .07 at 12 months ($^{*}P < .05$ vs. Wistar group at 12 months) in the SHR/High group (Fig 3, A, e-h and Fig 3, B). At age of 18 months, the staining intensity became much weaker in the SHR/Ve group than in the Wistar group. However, the SHR/Low and SHR/High groups showed considerable conservation of MAP2 staining compared with the SHR/Ve group (Fig 3, A, i, j, k, and l). The pixel intensities relative to that in the 6-month Wistar group were 1.21 ± .11 and .95 ± .20 at 18 months ($^{**}P < .01$ vs. Wistar group at 18 months) in the SHR/Ve group, 1.14 ± .08 at 18 months ($\#\#P < .01$ vs. SHR/Ve group) in the SHR/Low group, 1.25 ± .16 at 18 months ($\#\#P < .01$ vs. SHR/Ve group at 18 months) in the SHR/High group (Fig 3, B).

Immunohistochemical Analyses in the Hippocampus

LDL-R Staining in Hippocampus

In Wistar rats, LDL-R was scarcely labeled in the CA1 and CA3 neurons throughout 6-18 months of age (Fig 4, A, a, e,

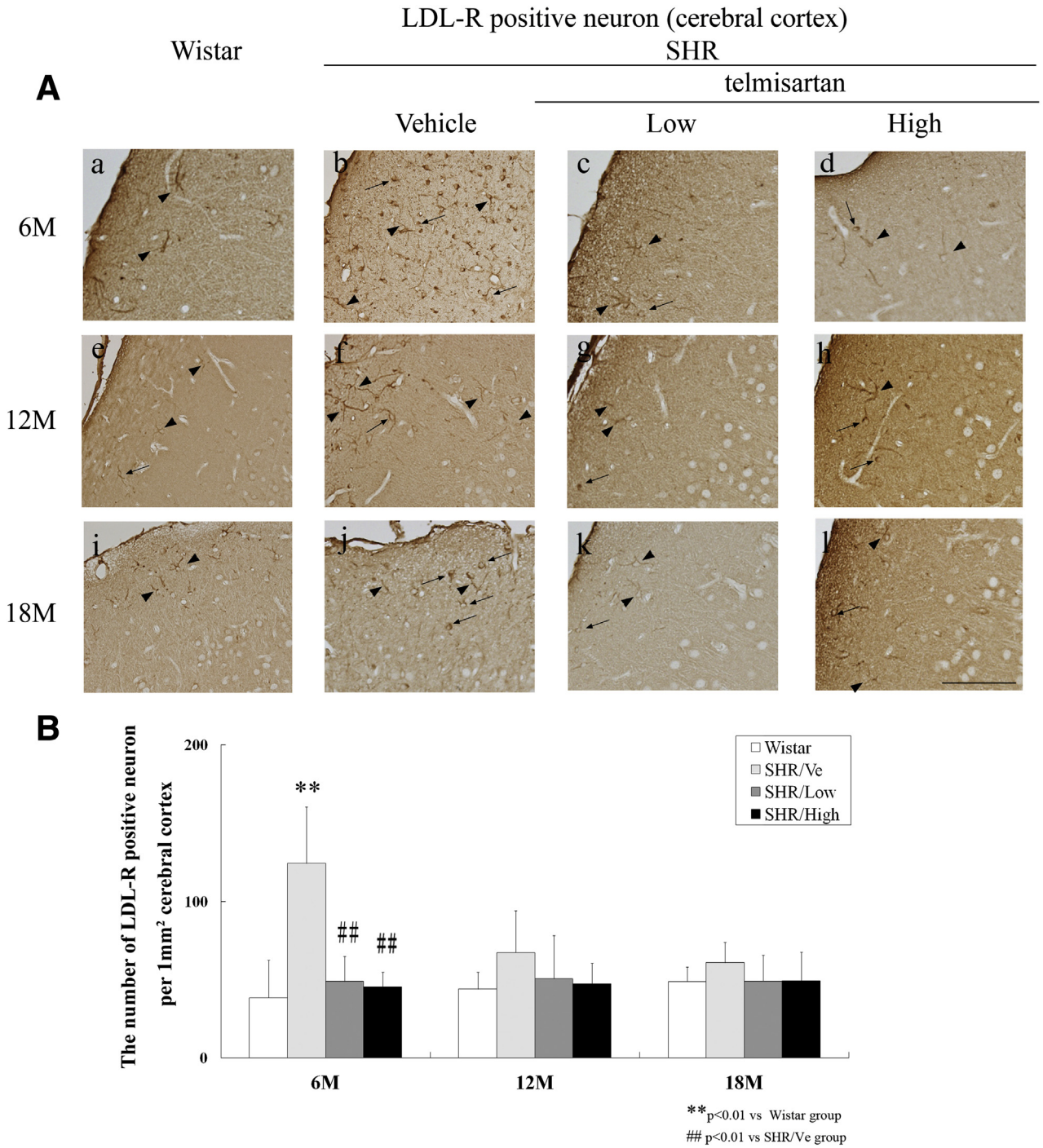

Figure 1. *Representative photomicrographs of LDL-R–positive neuron staining (A, arrows = neuronal cytoplasm, arrowheads = neuronal dendrite)and the numbers of LDL-R–positive neurons per 1 mm² of cerebral cortex (B) at ages 6, 12, and 18 months. Note the stronger staining in SHR/Ve group than Wistar group and the great reductions in the 2 telmisartan-treated groups. (Scale bar = 100 μm). Abbreviations: High, high-dose telmisartan-treated group; LDL-R, low-density lipoprotein receptor; Low, low-dose telmisartan-treated group; M, months; SHR-SR, spontaneously hypertensive rat stroke resistant; Ve, vehicle.*

and i). The ratio of LDL-R–positive to all neurons of CA1 and CA3 sectors were .09 ± .02 at 6 months, .09 ± .03 at 12 months, and .10 ± .09 at 18 months, and no change was detected with age in the Wistar group (Fig 4, B, open bars).

In the SHR/Ve group, LDL-R was clearly labeled in CA1 and CA3 neurons at 6 months (Fig 4, A, b, f, and j), but the ratio of LDL-R–positive to all neurons in hippocampus noticeably decreased with age from .49 ± .19 at 6 months (**$P < .01$ vs. Wistar group at 6 months) to .39 ± .24 at 12 months (*$P < .05$ vs. Wistar group at 12 months) and .17 ± .03 at 18 months (**$P < .01$ vs. Wistar group at 18 months; Fig 4, B).

In the 2 telmisartan-treated groups, LDL-R was weakly labeled in the cytoplasm of CA1 and CA3 neurons

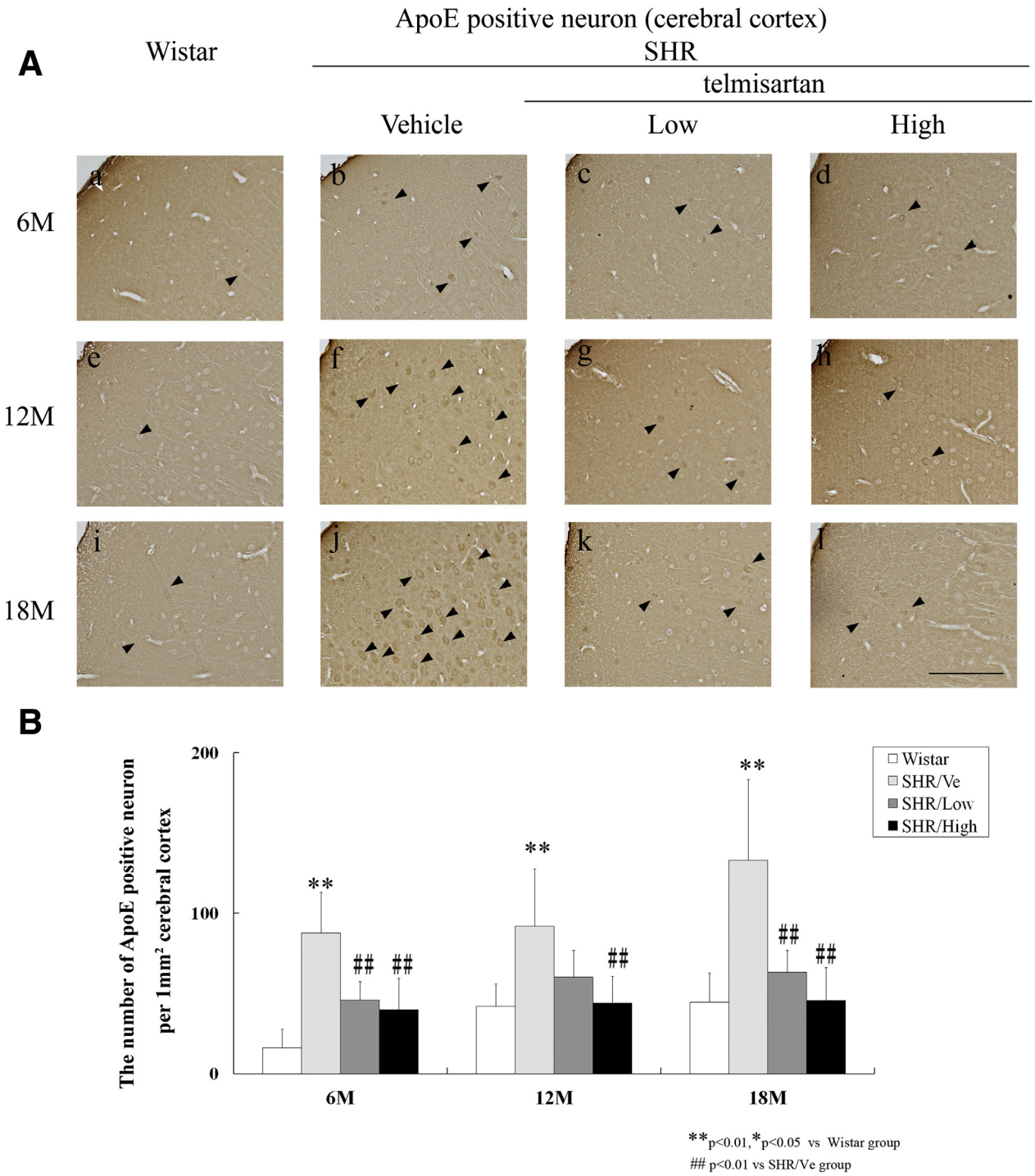

Figure 2. *Representative photomicrographs ApoE-positive neuron staining (A, arrowheads) and the numbers of ApoE-positive neurons per 1 mm² of cerebral cortex (B) at ages 6, 12, and 18 months. Note the stronger staining in SHR/Ve group than Wistar group and the great reduction in the 2 telmisartan-treated groups. (Scale bar = 100 μm). Abbreviations: ApoE, apolipoprotein E; High, high-dose telmisartan-treated group; Low, low-dose telmisartan-treated group; M, months; SHR-SR, spontaneously hypertensive rat stroke resistant; Ve, vehicle.*

throughout 6-18 months, similar to the level seen in the Wistar group (Fig 4, A, c, d, g, h, i, and k). The ratio of LDL-R positive to all neurons of CA1 and CA3 sectors in the SHR/Low group were .19 ± .05 at 6 months (##$P < .01$ vs. SHR/Ve group at 6 months), .12 ± .12 at 12 months, and .09 ± .03 at 18 months (##$P < .01$ vs. SHR/Ve group at 18 months), and those in the SHR/High group was .13 ± .05 at 6 months (##$P < .01$ vs. SHR/Ve group at 6 months), .11 ± .05 at 12 months (#$P < .05$ vs. SHR/Ve group at 12 months), and .08 ± .03 at 18 months (##$P < .01$ vs. SHR/Ve group at 18 months, Fig 4, B).

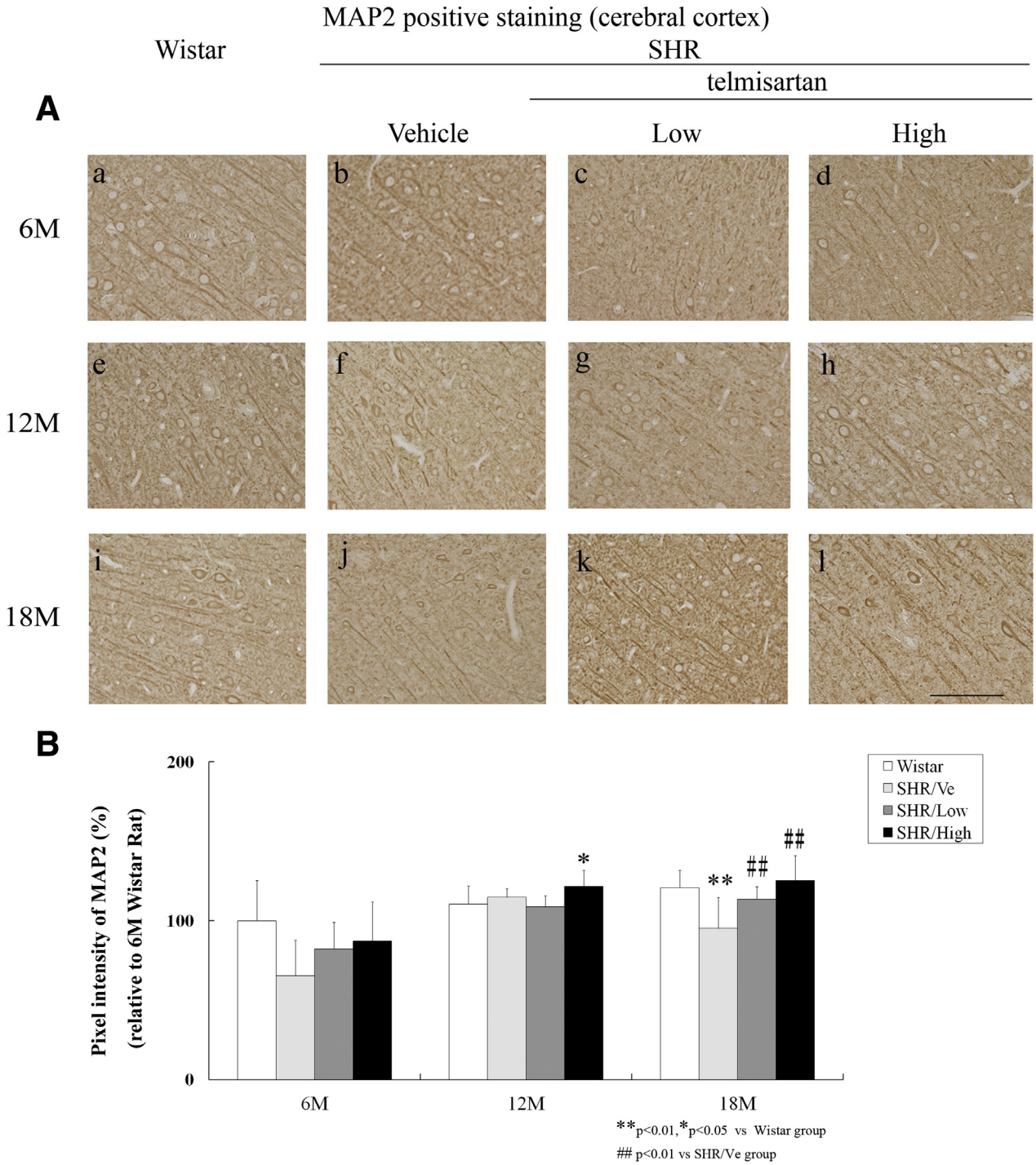

Figure 3. *Representative photomicrographs of MAP2-positive neuron staining (A) and the pixel intensity for MAP2 compared with the Wistar group in cerebral cortex (B) at ages 6, 12, and 18 months. Note the lower pixel intensity for MAP2 in the SHR/Ve compared with Wistar group at 18 months and the improvements in the 2 telmisartan-treated groups at 12 and 18 months. (Scale bar = 100 μm). Abbreviations: High, high-dose telmisartan-treated group; Low, low-dose telmisartan-treated group; M, months; MAP2, microtubule-associated proteins 2; SHR-SR, spontaneously hypertensive rat stroke resistant; Ve, vehicle.*

ApoE Staining in the Hippocampus

In Wistar rats, ApoE was scarcely labeled in CA1 and CA3 neurons at throughout 6-18 months of age, with no age-dependent change in the cytoplasm (Fig 5, A, a, e, and i). The ratio of ApoE positive to all neurons in CA1 and CA3 sectors were .03 ± .02 at 6 months, .05 ± .01 at 12 months, and .05 ± .02 at 18 months (Fig 5, B, open bars).

In the SHR/Ve group, ApoE was already labeled in CA1 and CA3 neurons at 6 months, and the ratio of

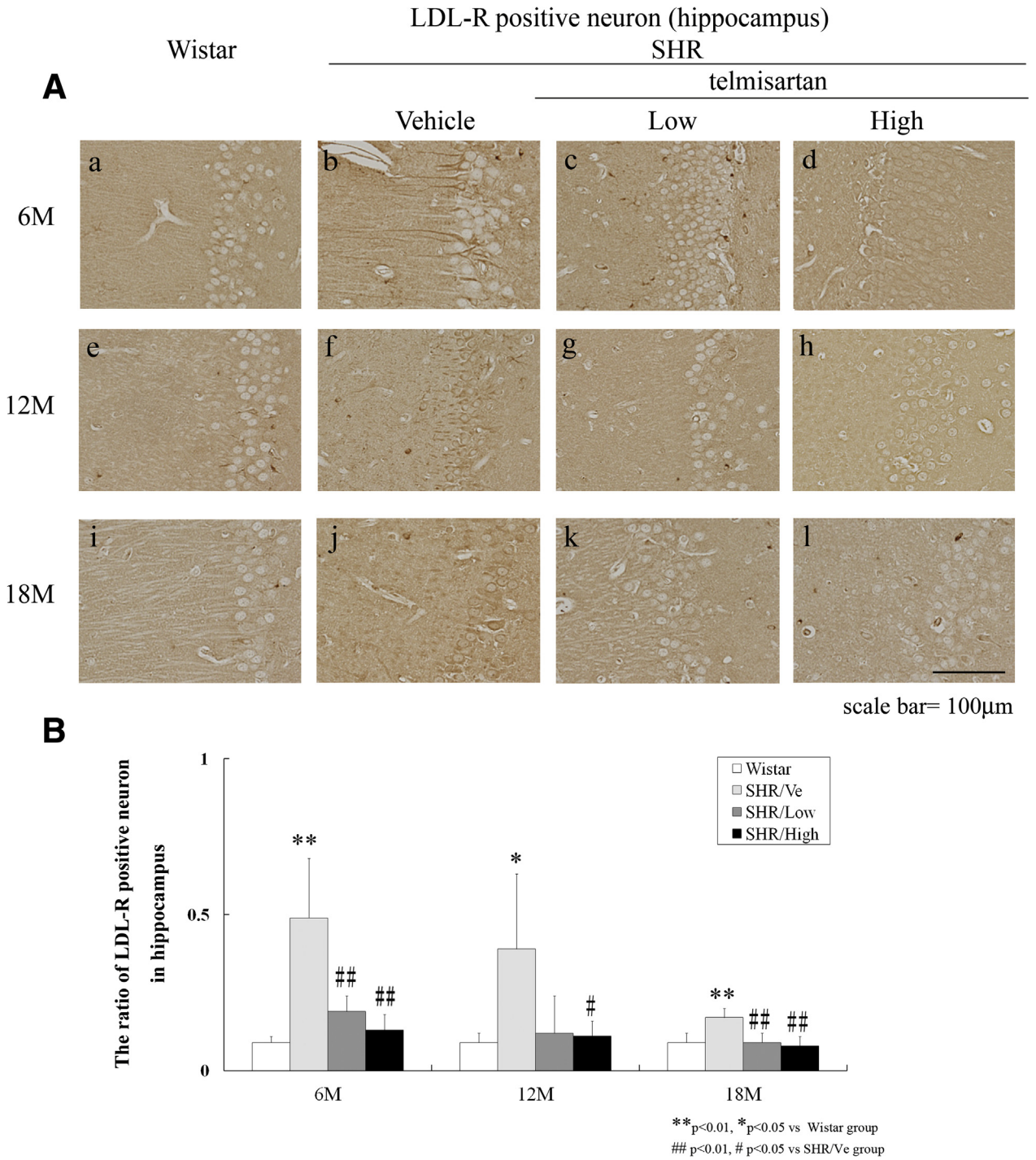

Figure 4. *Representative photomicrographs of LDL-R–positive neuron staining (A) and the ratio of LDL-R–positive neuron of the hippocampal CA1 sector (B) at ages 6, 12, and 18 months. Note the stronger staining in SHR/Ve group than Wistar group and the great reduction in the 2 telmisartan-treated groups. Abbreviations: High, high-dose telmisartan-treated group; LDL-R, low-density lipoprotein receptor; Low, low-dose telmisartan-treated group; M, months; SHR-SR, spontaneously hypertensive rat stroke resistant; Ve, vehicle.*

ApoE-positive neurons of CA1 and CA3 sectors obviously increased with age (Fig 5, A, b, f, and j). The ratio of ApoE-positive to all neurons of CA1 and CA3 were .16 ± .10 at 6 months (**$P < .01$ vs. Wistar group at 6 months), .20 ± .13 at 12 months (*$P < .05$ vs. Wistar group at 12 months), and .29 ± .16 at 18 months (**$P < .01$ vs. Wistar group at 18 months; Fig 5, B).

Similar to LDL-R, in the 2 groups of telmisartan-treated SHR-SR, LDL-R was weakly labeled in the cytoplasm of CA1 and CA3 neurons throughout 6-18 months of age, similar to the level seen in the Wistar group (Fig 5, A, c, d, g, h, i, and k). The ratio of LDL-R positive to all neurons of CA1 and CA3 sectors in the SHR/Low group were .07 ± .03 at 6 months, .07 ± .03 at 12 months, and

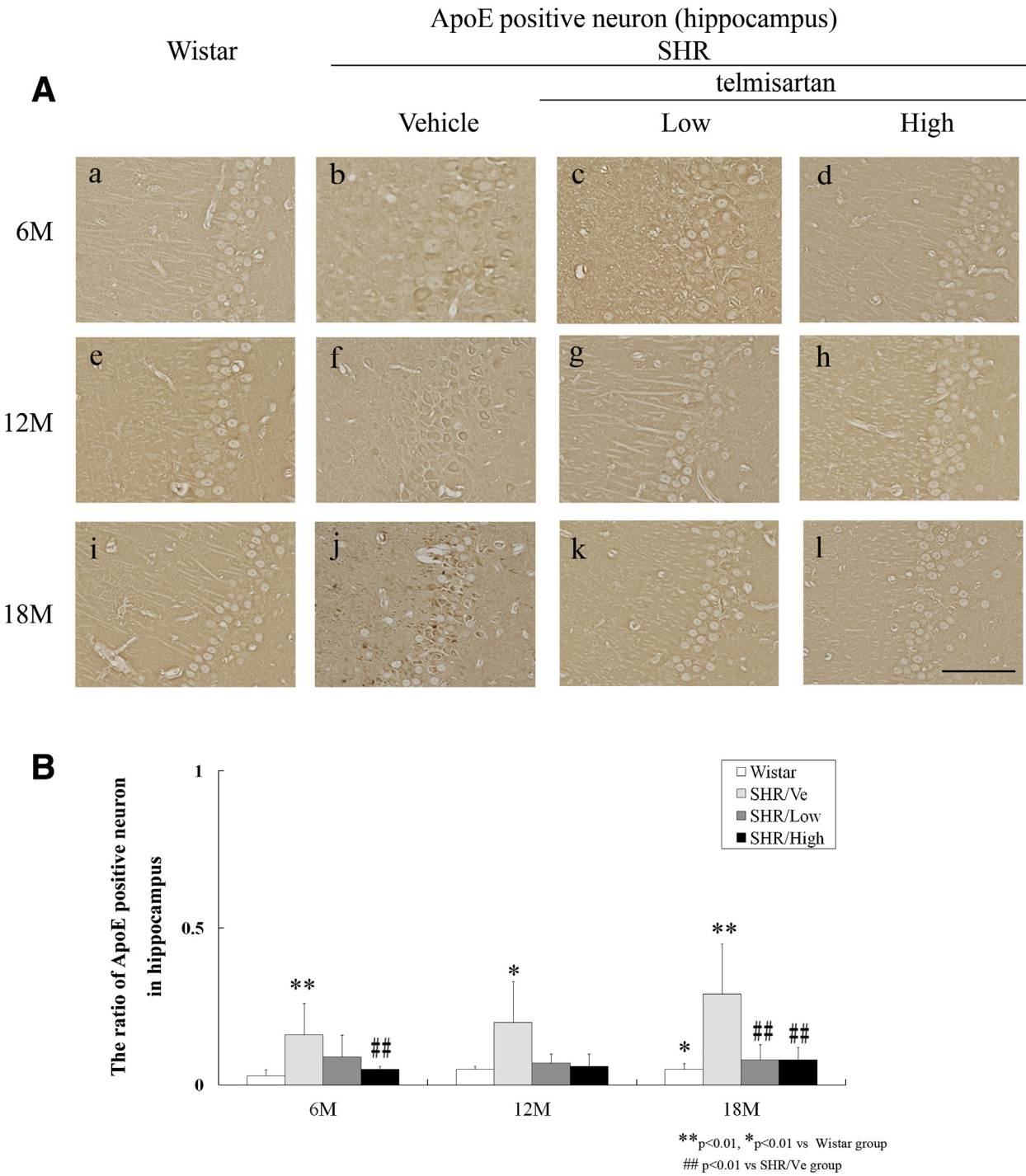

Figure 5. *Representative photomicrographs of ApoE-positive neuron staining (A) and the ratio of ApoE-positive neurons of the hippocampal CA1 sector (B) at ages 6, 12, and 18 months. Note the stronger staining in SHR/Ve group than Wistar group and the great reduction in the 2 telmisartan-treated groups. (Scale bar = 100 μm). Abbreviations: ApoE, apolipoprotein E; High, high-dose telmisartan-treated group; Low, low-dose telmisartan-treated group; M, months; SHR-SR, spontaneously hypertensive rat stroke resistant; Ve, vehicle.*

.08 ± .05 at 18 months (##$P < .01$ vs. SHR/Ve group at 18 months), and those in the SHR/High group was .05 ± .01 at 6 months (##$P < .01$ vs. SHR/Ve group at 6 months), .16 ± .04 at 12 months, and .08 ± .04 at 18 months (##$P < .01$ vs. SHR/Ve group at 18 months; Fig 5, B).

MAP2 Staining in the Hippocampus

MAP2 was detectable in the dendrites and spines of the CA1 and CA3 neurons throughout 6-18 months of age (Fig 6, A). Compared with age-matched Wistar group, the intensity of staining significantly decreased, related

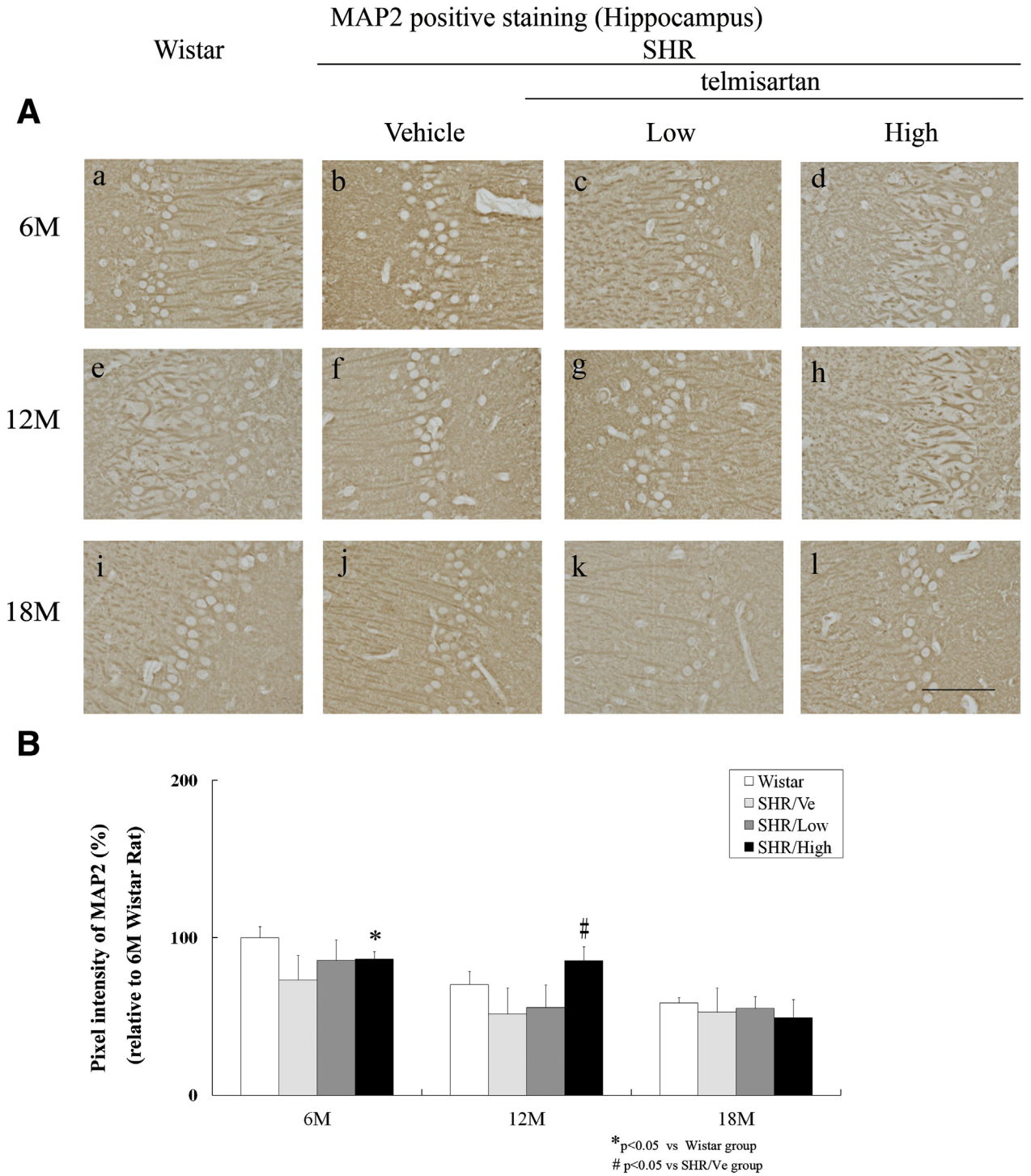

Figure 6. *Representative photomicrographs of MAP2-positive neuron staining (A) and the pixel intensity for MAP2 compared with the Wistar group in the hippocampal CA1 sector (B) at ages 6, 12, and 18 months. Note the lower pixel intensity for MAP2 in the SHR/Ve compared with Wistar group and the improvements only in the high-dose telmisartan-treated group at 6 and 12 months. Abbreviations: High, high-dose telmisartan-treated group; Low, low-dose telmisartan-treated group; M, months; MAP2, microtubule-associated proteins 2; SHR-SR, spontaneously hypertensive rat stroke resistant; Ve, vehicle.*

to age increasing in the SHR/Ve group and in the 2 groups of telmisartan-treated SHR-SR.

Quantitative analysis of pixel intensity for MAP2 showed that the pixel intensities relative to that in the 6-month Wistar group were .73 ± .16 at 6 months in the SHR/Ve group, .85 ± .13 at 6 months in the SHR/Low group, and .86 ± .05 at 6 months in the SHR/High group (*$P < .05$ vs. Wistar group at 6 months; Fig 6, A, a-d and Fig 6, B). At 12 months of age, the staining intensity became much weaker in the SHR/Ve group than in the

Wistar group, similar to the level seen in the SHR/Low group. However, the SHR/High groups showed considerable conservation of MAP2 staining compared with the SHR/Ve group (Fig 6, A, e-h). The pixel intensities relative to that in the 6-month Wistar group were .52 ± .17 at 12 months in the SHR/Ve group, .56 ± .14 at 12 months in the SHR/Low group, and .85 ± .09 at 12 months (#$P < .05$ vs. SHR/Ve group at 12 months) in the SHR/High group. At of 18 months of age, Wistar, SHR/Ve, and SHR/Low groups showed approximate conservation of MAP2 staining. The pixel intensities relative to that in the 6-month Wistar group were .53 ± .15 at 18 months in the SHR/Ve group, .55 ± .08 at 18 months in the SHR/Low group, and .49 ± .12 at 18 months in the SHR/High group (Fig 6, A, i-l and Fig 6, B).

Discussion

SHR-SR showed an abnormal lipid metabolism,[19,20] and telmisartan-improved serum levels of free fatty acids, triglycerides, and glucose in SHR-SR.[21] Our previous report showed that LDL-R is expressed in conjunction with ApoE receptor in the neuronal cytoplasm and dendrites after cerebral ischemia.[22] In the present study, we found that the numbers of LDL-R– and ApoE-positive neurons increased in both cerebral cortex and hippocampus of SHR/Ve throughout 6-18 months of age, compared with age-matched normotensive Wistar rats (Figs 1, 2, 4, and 5). However, telmisartan significantly reduced the numbers of LDL-R– and ApoE-positive neurons in both cerebral cortex and hippocampus, with similar effectiveness in the SHR/Ve group without BP lowering (SHR/Low) to BP lowering (SHR/High; Figs 1, 2, 4, and 5). Thus, attenuation of LDL-R/ApoE signals by telmisartan treatment may mainly be through its antimetabolic syndrome effect (Figs 1, 2, 4, and 5) with only a small additive effect by BP lowering (Figs 1, 2, 4, and 5). In this study, LDL-R expression was downregulated with age increasing both in the hippocampus and the cerebral cortex.

On the other hand, the numbers of MAP2-positive neuron were decreased with age increasing in the hippocampus, but the expression trend in the cerebral cortex was still stable. Kinoshita et al[23] reported that SHR-SR hippocampal CA1 was more vulnerable to oxidative stress. We also recently reported a persistent hypertension in SHR-SR, which strongly potentiates oxidative stress.[24] MAP2 is a neuron specific cytoskeletal protein enriched in dendrites and cytoplasm, and the decrease is supposed as an early marker of ischemic brain injury.[22,25] Therefore, cerebral oxidative stress might injure hippocampal CA1 neurons in the SHR-SR. Previous reports indicated MAP2 can promote microtubule assembly and stability by crosslinking microtubule networks in dendritic compartments.[26,27,28] Targeted disruption of MAP2 decreases microtubule bundling, reduces dendritic microtubule density, and impairs dendritic elongation, leading to learning and memory impairment.[29,30,31] The numbers of MAP2-positive neuron were decreased in the cerebral cortex of SHR/Ve at 18 months, compared with normotensive Wistar rats (Fig 3), but such decrease was recovered by telmisartan in the cerebral cortex at 18 months (Fig 3) and the hippocampus at 12 months (Fig 6). Accumulating evidence showed that antihypertensive treatment prevented a cognitive decline,[32,33] and treatment with angiotensin II type 1 receptor blockers preserved cognitive function through a mechanism beyond the BP reduction.[34] The present study showed the recovery of MAP2 expression levels with telmisartan treatment even without lowering BP at 18 months (Fig 3). Thus, telmisartan can protect neurons in the SHR-SR rats possibly as a result of antimetabolic effect.

In the present study, cerebral cortex and hippocampus showed the similar patterns of LDL-R, ApoE, and MAP2 expressions (Figs 1-6), indicating that telmisartan did attenuate ApoE/LDL-R activity and enhance MAP2 expression not only in the cerebral cortex but also in the hippocampus. In addition to hypertension, the SHR model is also characterized by cognitive impairment.[35] We observed that SHR-SRs displayed a decrease in MAP2 immunostaining in the CA1 and CA3 sectors of hippocampus (Fig 6), the area where the LDL-R/ApoE is particularly enriched in SHR/Ve group (Figs 4-6), suggesting that abnormal lipid metabolism (Figs 4, A, b, f, and j and Fig 5, A, b, f, and j) could affect the hippocampal neurons relating to impaired cognitive function. As previous reports demonstrated, ApoE promoted accumulation of both Abeta plaques[36,37] and tau[38-41] and is colocalized with Abeta, cholesterol, and cholesterol oxidase.[7] In the present study, we found that telmisartan-treated SHR-SRs showed a significant decrease in LDL-R– and ApoE-positive neurons in the CA1 and CA3 sectors of hippocampus (Figs 4-6). As previous reports demonstrated, a significant subset of the actions of PPARγ activation has been reported to be involved in lipid metabolism and reverse cholesterol transport, including ApoE.[14] Thus, in the present study, we speculated that the protective effects of telmisartan improve the LDL-R/ApoE system partially through PPARγ activation.

In summary, the present data strongly suggest that telmisartan improved lipid metabolism as revealed by the presence of LDL-R and ApoE and protected the neurons in the brains of SHR-SR both in the cerebral cortex and hippocampus. Because telmisartan has a long-term neuroprotective effect to inhibit metabolic syndrome, it could provide a preventative approach in patients with hypertension at risk of AD.

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