Original Research

Ginkgolide B promotes oligodendrocyte precursor cell differentiation and survival via Akt/CREB/bcl-2 signaling pathway after white matter lesion

Jian Huang[1,*], Jun Yang[2,*], Xingju Zou[1], Shilun Zuo[3], Jing Wang[1], Jing Cheng[1], Hao Zhu[1], Weiwang Li[1], Ming Shi[1], Gang Zhao[1] and Zhirong Liu[1]

[1]Department of Neurology, Xijing Hospital, Airforce Military Medical University, Xi'an, Shaanxi 710032, China; [2]Department of Nephrology, Xijing Hospital, Airforce Military Medical University, Xi'an, Shaanxi 710032, China; [3]Department of Neurology, Second Affiliated Hospital of Army Military Medical University, Chongqing 400038, China

Corresponding authors: Zhirong Liu. Email: liuzhir8019@126.com; Gang Zhao. Email: zhaogang@fmmu.edu.cn; Ming Shi. Email: biomidas@fmmu.edu.cn

*These authors contributed equally to this paper.

Impact statement

Ginkgolide B has a well-established role of neuroprotective effect that could be beneficial for the treatment of ischemia. Our study demonstrated that ginkgolide B could promote oligodendrocyte precursor cell (OPC) differentiation into oligodendrocytes and promote oligodendrocyte survival following a white matter lesion. In addition, ginkgolide B promotes rats' learning and memory ability after white matter lesion (WML), reduces the myelin loss, promotes OPC differentiation, and attenuates the apoptosis. We analyzed the underlying mechanisms, including changes in membrane protein level of p-Akt, p-CREB, and Bcl-2. The findings show that ginkgolide B has a central role in promoting OPC differentiation and oligodendrocyte survival following chronic cerebral hypoperfusion by means of the Akt/CREB/Bcl-2 signaling pathway, and so ginkgolide B could potentially be a promising therapeutic agent for WML.

Abstract

White matter lesion (WML) is caused by chronic cerebral hypoperfusion, which are usually associated with cognitive impairment. Evidence from recent studies has shown that ginkgolide B has a neuroprotective effect that could be beneficial for the treatment of ischemia; however, it is not clear whether ginkgolide B has a protective effect on WML. Our data show that ginkgolide B can promote the differentiation of oligodendrocyte precursor cell (OPC) into oligodendrocytes and promote oligodendrocyte survival following a WML. Ginkgolide B (5, 10, 20 mg/kg) or saline is administered intraperitoneally every day after WML. After 4 weeks, the data of Morris water maze suggested that rats' memory and learning abilities were impaired, and the administration of ginkgolide B enhanced behavioral achievement. Also, treatment with ginkgolide B significantly attenuated this loss of myelin. Our result suggests that ginkgolide B promotes the differentiation of OPC into oligodendrocytes. We also found that ginkgolide B ameliorates oligodendrocytes apoptosis. Furthermore, ginkgolide B enhanced the expression of phosphorylated Akt and CREB. In conclusion, our data firstly show that ginkgolide B promotes oligodendrocyte genesis and oligodendrocyte myelin following a WML, possibly involving the Akt and CREB pathways.

Keywords: Ginkgolide B, oligodendrocyte precursor cells, white matter lesion, apoptosis, Akt

***Experimental Biology and Medicine* 2021; 246: 1198–1209. DOI: 10.1177/1535370221989955**

Introduction

Oligodendrocytes are known to encase axonal processes and assemble compact myelin, thereby forming cerebral white matter.[1,2] White matter lesion (WML) is often seen in aging, stroke.[3–5] WML is usually the cause of cognitive impairment, usually caused by chronic cerebral hypoperfusion.[6,7] The neuropathologic features of cerebral WML are: comprehensive apoptosis of oligodendrocytes, demyelination, and axonal injury.[8–11]

However, the internal mechanism of these changes has not been well studied. Multiple experiments have shown that inflammation is critical in the initiation of WML.[12–14] Thus, relieving WML is of great significance for the apoptosis of oligodendrocytes after inflammatory injury. Moreover, in some cases, OPCs may differentiate to mature oligodendrocytes that constitute myelin sheath.[15–17] Apoptosis or myelin sheath injury of oligodendrocytes may lead to rapid proliferation of oligodendrocyte

progenitor cells and migration to demyelinating zone, thereby differentiating into mature oligodendrocytes and forming new myelin sheath.[18–20] Therefore, these results indicate that any drug may achieve the therapeutic effect of WML by reducing apoptosis of oligodendrocyte and OPC can differentiate into mature oligodendrocyte.

Ginkgolide B can improve atherosclerosis by inhibiting platelet activation in the PI3K/Akt pathway.[21] Ginkgolide B is able to penetrate cerebral blood–brain barrier, particularly following cerebral ischemia–reperfusion damage, which may produce a significant effect to treat cerebral ischemia–reperfusion injury.[22] The electrical activity of paraventricular neurons can be inhibited by ginkgolide B.[23] Ginkgolide B may inhibit the inflammatory reaction of TLR-4 and NF-κ B, and alleviate the apoptosis of neuron after traumatic brain injury (TBI), which shows the therapeutic effect of ginkgolide B on TBI.[24] Ginkgolide B can enhance endothelial progenitor cells through the Akt signaling pathway.[25] Ginkgolide B is able to precondition against the apoptosis induced by ischemia, and its mechanism could be related to the phosphatidylinositol 3 kinase (PI3K) signal pathway.[26]

However, whether ginkgolide B can promote OPC differentiation and survival has not been reported. First of all, the rat WMLs model was established by blocking bilateral common carotid arteries. Next, our study focused on whether the protection of ginkgolide B against WML induced by means of cerebral hypoperfusion was related to the anti-apoptotic effect by activating Akt phosphorylation. Finally, our experiments showed that ginkgolide B promotes OPC differentiation by activating Akt/CREB/Bcl2 signaling pathway.

Materials and methods

Animals treatment and experiments design

All Sprague-Dawley (SD) rats described in the research were allowed through the Animal Research Committee of Airforce Military Medical University. All animals were nursed using the guidelines for animal use and nurse issued through National Institutes of Health Guide. SD rats, which were adult males and weighed between 280 and 320 g, were raised in a standardized laboratory and were given free food and water. We randomly divide SD rats into four groups: normal, control, ginkgolide B and LY294002 + ginkgolide B group. LY294002 + ginkgolide B group was only used in the mechanism study of ginkgolide B. In addition to Morris water maze and anxiety-related behavior experiments, 12–15 rats were used in each group, and 3–5 rats were used in other experiments. The WML model was constructed through blocking bilateral common carotid artery using the previously represented method.[27,28] The body temperature of SD rats was held at $37 \pm 0.5\,^{\circ}C$ during chronic cerebral ischemia operation. Rats in control, ginkgolide B, and LY294002 + ginkgolide B groups underwent the operation. Rats in the ginkgolide B group were given different doses (5, 10, 20 mg/kg) of ginkgolide B (Sigma, USA), which is soluble in normal saline by intraperitoneal injection every day for 4 weeks after operation. The control group was given equal amount of distilled water after operation. Those in the LY294002 + ginkgolide B group received 5 mg/kg of ginkgolide B after operation. The final concentration of PI3K inhibitor LY294002 (5 μl, Cell signaling, USA) was 10 mM, which is soluble in dimethyl sulfoxide, through injecting into the lateral ventricle with microinjector 1 h before operation, as described previously.[29]

Morris water maze experiment

Morris water maze trial was executed using previous method.[30] The purpose of all experiments was to assess the spatial learning and memory level of SD rats. There is a circular platform submerged under the water. All experimental sessions were performed between 09:00 and 12:00. The navigation trial was repeated four times daily for 5 days. The animals were tested on a set of semi-random starting positions. The starting position of the far end in the pool must be equal to the length of the target, and the time of each experiment is 60 s. If the SD rat cannot seek out the platform at a certain time, the experimenter guides it to the target and places it on the platform for 15 s. The escape latency is calculated by the time it takes the animal to successfully seek out the platform. The time of each arrival at the platform will be recorded. A 60 s probe experiment was implemented 24 h after the last training day to assess spatial memory retention. The platform needs to be removed during the probe test. In the probe experiment, the experimenter recorded the percentage of time spent in the target quadrant and the distance traveled in SD rats.

Anxiety and depression experiment

The open field trial was conducted in an independent, quiet square site with a total of 3600 square centimeters, with white floors in the center and black glass walls of 25 cm high all around. There were 36 small squares of the same size among them. Twenty small squares close to the glass walls were protected sites, or "arena periphery," and the remaining 16 were exposed fields, or "arena centers." The experimenters placed each rat in the middle of a square and the camera monitored it for 15 min, recording how long each rat stayed in the middle of the arena. The elevated plus trial was initiated with the method described earlier.[31] The experimenters placed each rat in the middle of the elevated plus maze with SD rat head facing the open arm. The camera above the maze continuously monitored the rats for 5 min, recording the visit times to the open and closed arms.

Histology and electron microscopy

To assess histologic changes after WML, hematoxylin and eosin (H&E) was used, as previously described.[32,33] Briefly, brains were removed and fixed in 4% paraform. The rat brain was sliced into 30 μm coronal slices before staining with H&E. After dehydration, the slices were washed and observed under a microscope. Luxol Fast Blue (LFB) was carried out as previously described[34] to assess the degree of demyelination. The rat brain slices were immersed overnight in 0.1% LFB liquid (Sigma, USA) at $37\,^{\circ}C$, and then

the dyes were removed successively with 95% ethanol, 0.05% lithium carbonate liquid, 70% ethanol and distilled water. After dehydration, the slices were fixed with gum and observed under a microscope. Demyelination was assessed using the previous methodology, from 0 to 3 (no demyelination to complete demyelination).[35] Before scanning electron microscope, the corpus callosum of rats which had been embedded with Epon and fixed with glutaraldehyde should be trimmed and repositioned in order to obtain a more ultrathin and accurate image. Myelin sheath thickness, diameter of fiber and axon were analyzed by Docu System. G ratios are the axons diameter divided by fibers diameter (sum of axon diameter and myelin sheath thickness). All G ratios, which were calculated as the G ratio for each fiber from one brain, were averaged. The proportion of axons with diameter $\geq$400 nm in all axons was counted as myelinated fibers.

TUNEL assay

Briefly, rinsed rat brain sections were soaked in osmotic solution for 2 min on ice, and the sections were rinsed and reacted with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay (TUNEL) Kit at 37 °C for 1 h. The washed sections reacted with Cy3-conjugated streptavidin for 60 min. The images were scanned by FV1000 confocal microscope (Olympus, Japan).

OPC culture

OPC was cultured and purified by the previous research methods.[36] In order to observe the differentiation of OPC more clearly, the experimenters cultured OPCs in modified OPC growth-medium. OPC was cultured with ginkgolide B (0.1, 1.0, 10, and 100 μM) and equal volume of normal saline for 3 days. Then 4% paraformaldehyde was applied to fix the OPCs for 30 min before immunocytochemistry.

Immunofluorescence staining

In Leica CM1900 cryostat (Germany), a 30 μm coronal section was cut at the corpus callosum level (1.20 to 5.04 mm posterior from bregma) in rats. First, rat slices were cultured all-night at 4 °C in PBS with anti-APC (1:100, Calbiochem, USA). The rinsed sections were cultured at room temperature with the second antibody combined with Cy3 (1:200, Vector) for 2 h. Hoechst 33342 (Sigma, USA) was applied to contrast the cell nuclei. The pictures were scanned using a confocal microscope (Olympus, Japan). OPCs were cultured all-night at 4 °C in PBS with anti-CNPase (1:500, Abcam). The rinsed OPCs were cultured at room temperature with the second antibody combined with Cy3 (1:300; Vector) for 1 h. Hoechst 33342 (Sigma, USA) was applied to contrast the cell nuclei. The pictures were scanned by means of a Leica microscope.

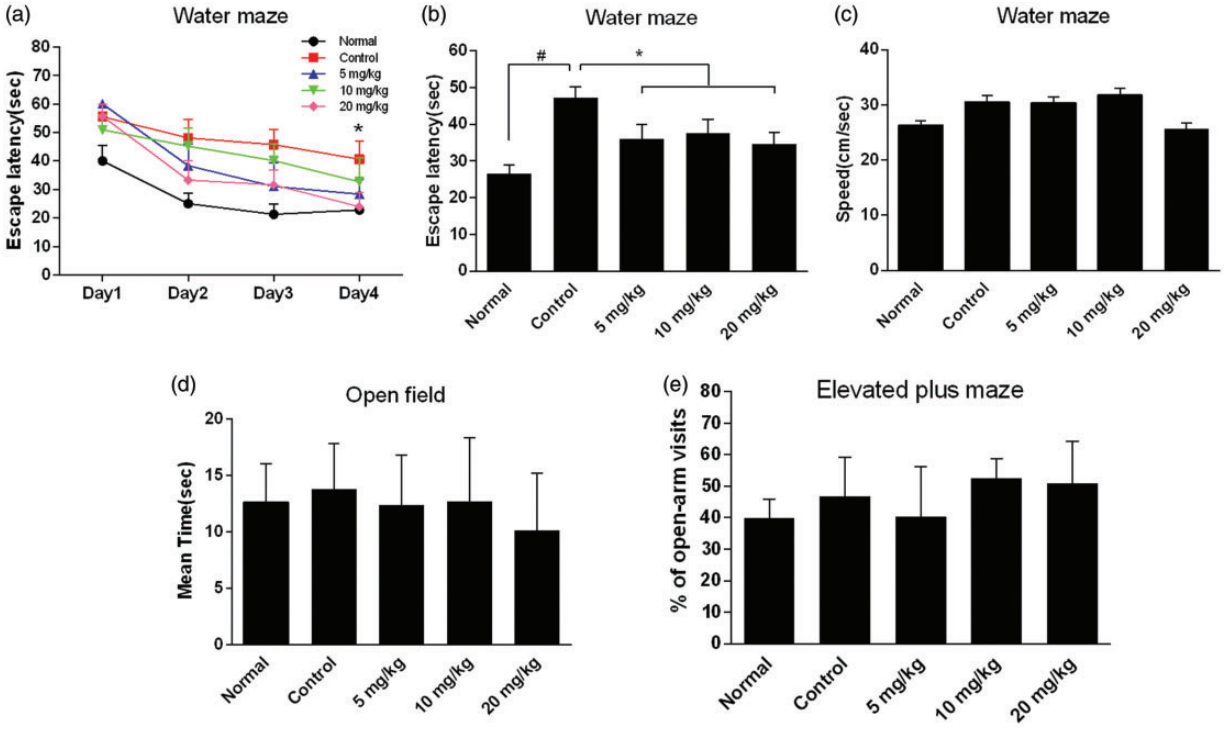

Figure 1. Learning in Morris water maze is impaired in WML rats and is ameliorated by treatment with ginkgolide B. (a to e) Spatial learning and memory ability in the Morris water maze experiment was assessed in normal and control rats, treated with ginkgolide B (5, 10, 20 mg/kg). (a) The escape latency of rats treated with ginkgolide B on the fourth day of training was significantly affected. (b) The escape latency of the fourth training day showed no conspicuous difference between ginkgolide B treatment SD rats. Data are the mean ± SEM of 12–15 rats each group. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats. (c) The swimming speed of rats in each group had no significant effect. (d and e) Data showed that no conspicuous difference existed among the ginkgolide B, control, and normal rats on anxiety and depression by (d) open field trial and (e) elevated plus experiments. (A color version of this figure is available in the online journal.)

Western blot analysis

We used the previous methods to conduct western blot experiments on the corpus callosum of rats and OPC cells cultured in vitro.[37] Briefly, Millipore's protein extraction kit is used for total protein extraction. The proteins were electrophoretic, transmembrane, and reacted with the first antibody overnight: anti- MBP (1:1000, Santa Cruz), anti-PDGFαR (1:500, Abcam), anti-CNPase (1:1000, Abcam), anti-GAPDH (1:3000, Kangwei), anti-cleaved caspase-3, anti-p-Akt, anti- p-CREB and anti-bcl-2 (1:1000, Cell signaling). The rinsed protein reacted with the second antibody (1:10,000, Kangwei) for 1 h, and then detected with the kit (Kangwei). The gray value of the stripe was quantitatively analyzed by Image J (NIH). GAPDH was analyzed as a control in all bands. The gray values of p-Akt and p-CREB were statistically analyzed with Akt and CREB, respectively.

Statistical analysis

APC positive cells were quantified by calculating immune labeled cells per square millimeter under a microscope (400 times magnification). At least six sections of each rat were used for statistical analysis. The area of cell count was concentrated in the middle of the corpus callosum of the rats. The CNPase positive cells were quantified by counting immune labeled cells per square centimeter under a microscope (200 times magnification). Statistical analysis was conducted through three to five times of experimental results. Results were showed mean ± SEM and assessed by SPSS 16.0 software. One-way ANOVA and Tukey's post hoc test were used to study the marked differences among groups in behavioral experiments and Western blot. Student's *t*-test was applied to investigate differences among ginkgolide B and the control groups in vitro experiment. For a P value < 0.05, the difference is deemed to be statistically significant.

Results

Ginkgolide B promotes rat learning and memory ability after WML

To investigate whether ginkgolide B can ameliorate learning and memory following WMLs, we performed the

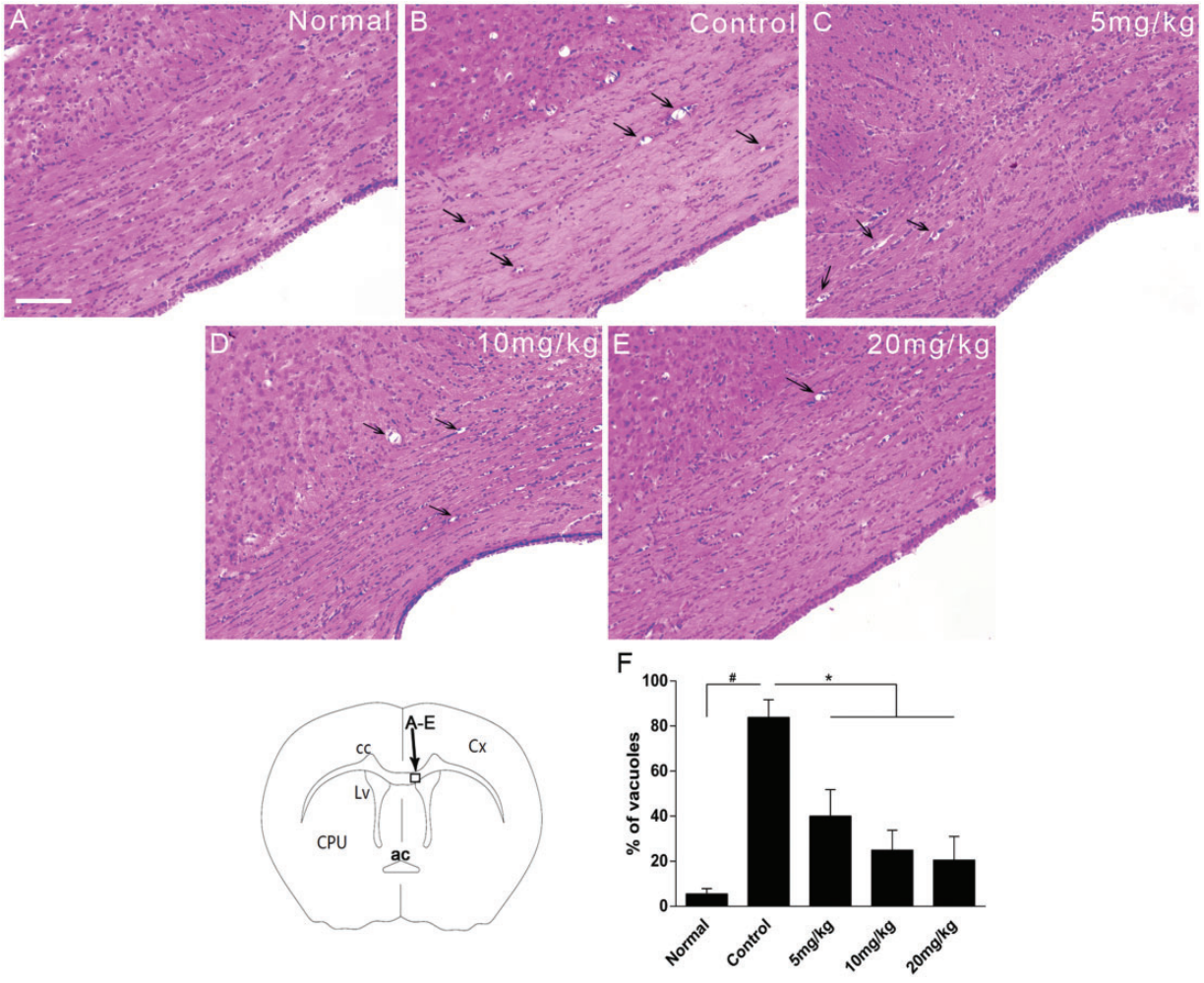

Figure 2. Corpus callosum (cc) rarefaction and vacuolation was more prominent in the brains of rats after WML. (a to e) We observed ginkgolide B attenuates white matter rarefaction and vacuolation after WML, using H&E staining. (a) Normal rats, (b) control rats, (c to e) 5–20 mg/kg ginkgolide B treatment of WML in rats. (f) Quantitation of the percentage of vacuoles to the total corpus callosum provided from the brain of SD rats following WMLs. Data are the mean ± SEM of six sections each rat and five rats each group. #p< 0.05 vs. normal rats. *p< 0.05 vs. control rats. Scale bar = 50 μm.

Morris water maze experiment. The results indicated that the escape latency of rats after WML was conspicuously longer than that of normal rats (Figure 1a). In addition, ginkgolide B treatment group rats were no different from those that were treated with NaCl for the first 3 days of training. Nevertheless, rats that were treated with ginkgolide B (5, 10, 20 mg/kg) took less time to seek out the platform on the fourth day after training (Figure 1b). However, none of the different dose ginkgolide B treatment groups were conspicuously different. Moreover, no conspicuous difference existed in swimming speed among all rats on four training days (Figure 1c). To determine whether ginkgolide B has an effect on anxiety and depression, open field and elevated plus maze experiments were applied to exclude behavior performance. The data indicated that no conspicuous difference existed among the ginkgolide B, control, and normal rats spent in middle of the stadium in the open field experiment (Figure 1d) and open-arm access ratio in the elevated plus experiment (Figure 1e). Overall, these results imply that ginkgolide B can enhance learning and memory ability following WMLs.

Ginkgolide B reduces the myelin loss

We used H&E staining to evaluate the histological changes of cerebral white matter following WMLs in rats. Our results suggest that in control group, the rarefaction and vacuolation of cerebral white matter was more obvious than in normal rats (Figure 2a and b). However, the effect was inverted by doses of 5–20 mg/kg of ginkgolide B groups (Figure 2c to f). To further examine the damage to the myelin sheaths, we performed LFB staining. In control rats, the staining demonstrated that myelin loss was apparent than in the normal group (Figure 3a and b). In sharp contrast, the effect was reversed by the ginkgolide B treatment (Figure 3c to f). To assess the degree of myelin loss at the ultrastructural level, Epon-embedded tissue of the corpus callosum was analyzed. The electron microscopy examinations demonstrated that, when compared with normal rats, myelinated axons and myelin thickness were greatly decreased in the control rats (Figure 4a and b). The effect was reversed by ginkgolide B administration (Figure 4c to i). Thus, these results indicate that ginkgolide

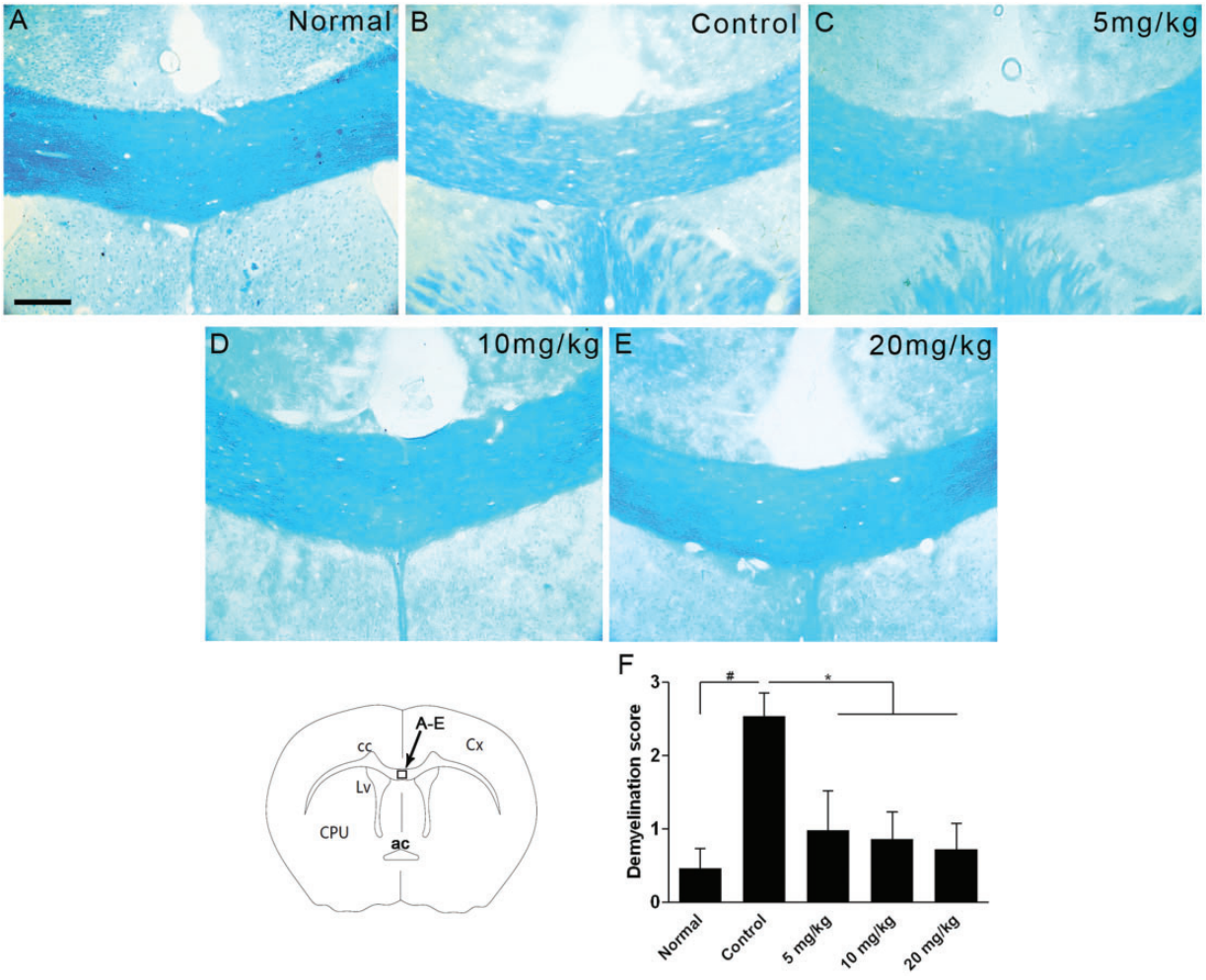

Figure 3. Corpus callosum (cc) demyelination was more prominent in the brains of rats after WML. (a to e) We observed ginkgolide B attenuates white matter rarefaction and vacuolation after WML, using Luxol Fast Blue (LFB) staining. (a) Normal rats, (b) control rats, (c to e) WML rats treated with 5–20 mg/kg ginkgolide B. (f) Quantitation of demyelination of corpus callosum provided from the brain of SD rats following WMLs. Data are the mean ± SEM of six sections per rat and five rats each group. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats. Scale bar = 100 μm.

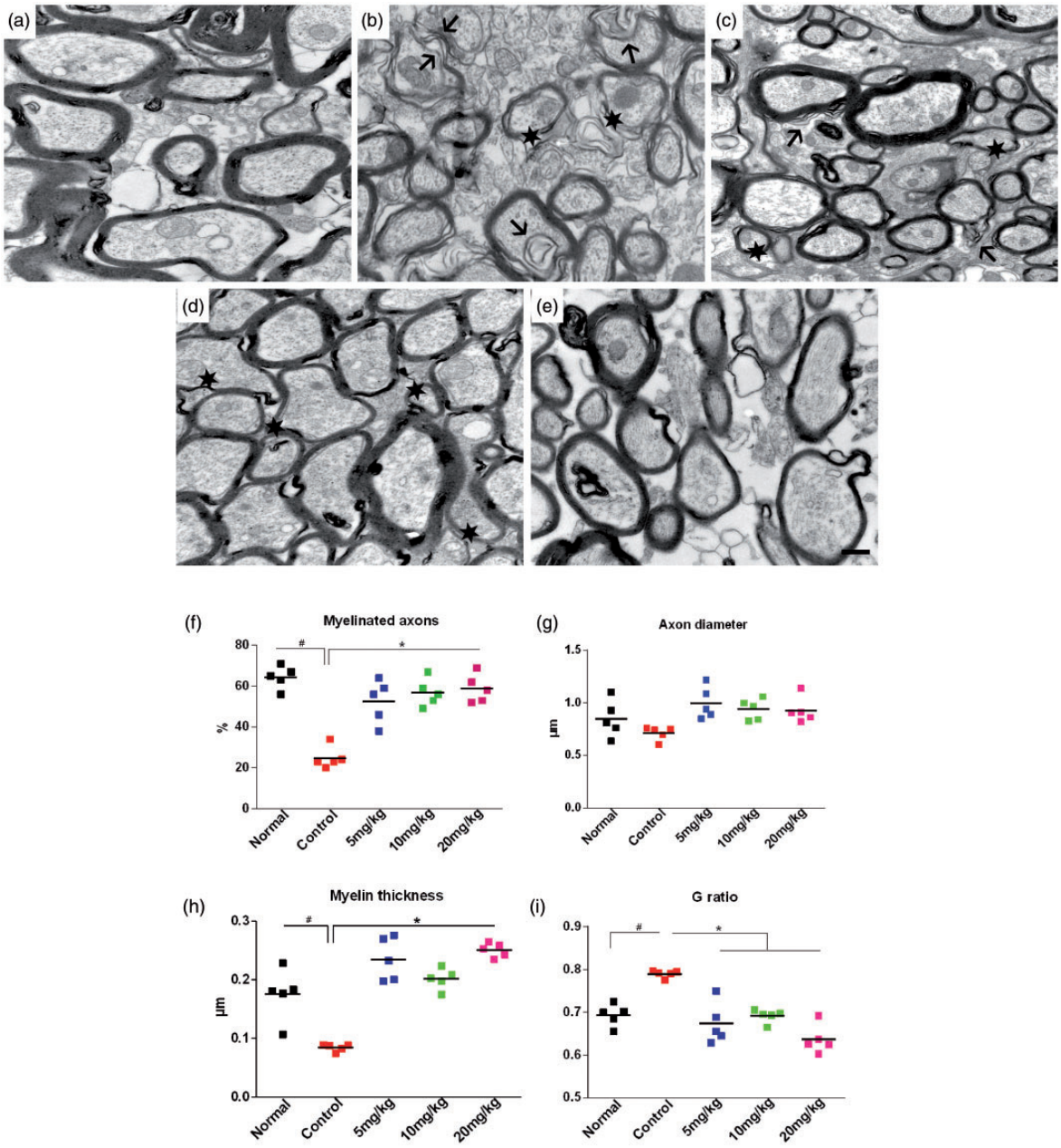

Figure 4. Corpus callosum (cc) demyelination was more prominent in the brains of rats after WML. Ginkgolide B promotes myelin thickness after WML. (a) Normal group, (b) control group, and (c to e) different concentrations of ginkgolide B treatment group after 4 weeks of electron microscopic images (40,000 ×). Demyelination is represented by arrows and myelination thinning by asterisks. Scale bar: 0.5 μm. (f to i) demonstrated quantitation of the percentage of (f) myelinated axons to the total axons in each group, (g) axon diameter, (h) myelin thickness, (i) *G*-ratio at 4 weeks. Data are the mean ± SEM of six sections each rat and five rats each group. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats.

B may protect against chronic cerebral hypoperfusion by suppressing histopathological changes and attenuating myelin loss.

Ginkgolide B promotes OPC differentiation

To examine whether ginkgolide B has an effect on oligodendroglia in WMLs in rat brains, we used the changes of mature oligodendrocytes to evaluate WML. The APC positive oligodendrocytes in WML rats were conspicuously lower than that in the normal group (Figure 5a and b). However, ginkgolide B treatment ameliorated the decrease of oligodendrocytes after cerebral ischemia (Figure 5c to f). The expression of PDGFαR was upregulated by western blot following WML and was decreased through the treatment of ginkgolide B (Figure 6b). In sharp contrast, ischemia induced the downregulation of MBP expression (Figure 6a), which was reversed by ginkgolide B treatment. Interestingly, ginkgolide B treatment did not affect CNPase expression (Figure 6c). Then, in order to investigate whether ginkgolide B can increase mature oligodendrocytes in vitro, the cultured OPCs were incubated with ginkgolide B for 3 days. The data indicated that, when compared to the control, more CNPase positive cells were examined in

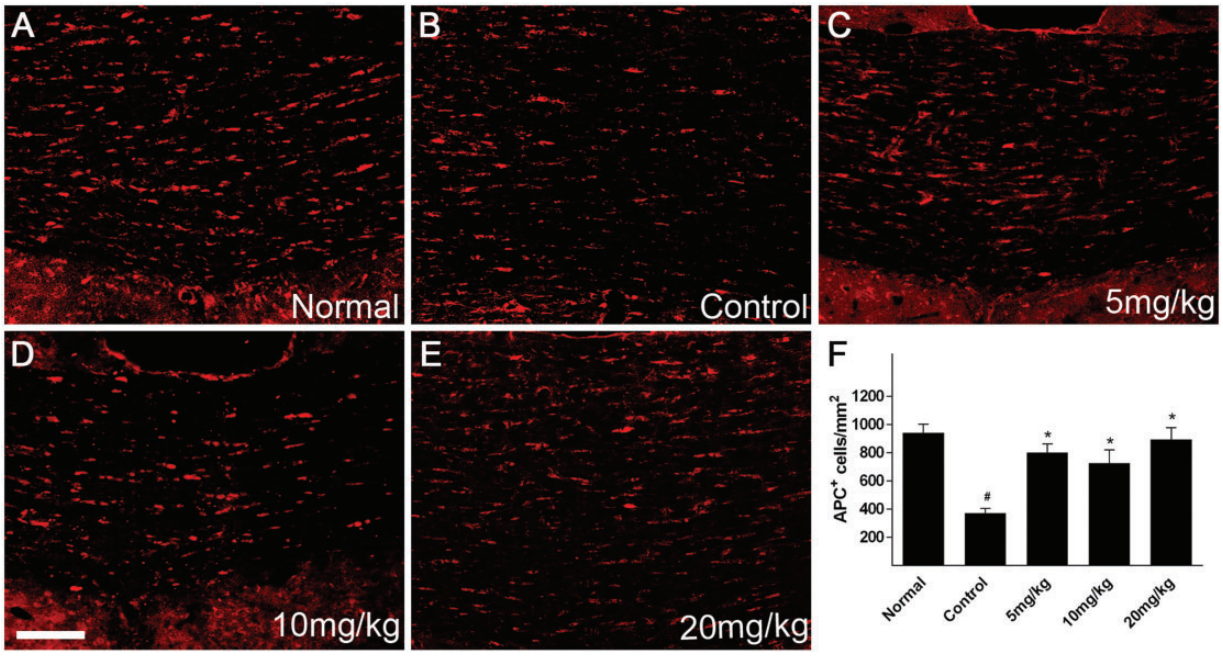

Figure 5. Ginkgolide B increases survival of oligodendrocytes following WMLs. (a) Normal, (b) control, and (c to e) 5–20 mg/kg ginkgolide B-treated rats of immunofluorescence staining of oligodendrocytes. Data are mean ± SEM of six sections each rat and five rats each group. (f) Quantitative analysis of APC + cells in each group. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats. Scale bars = 50 μm. (A color version of this figure is available in the online journal.)

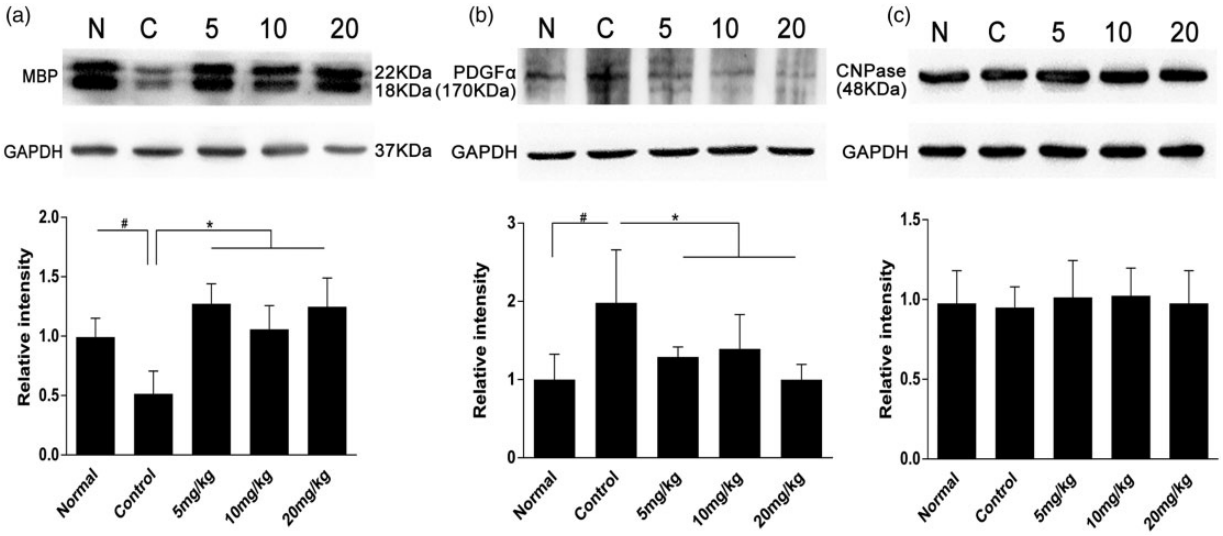

Figure 6. Ginkgolide B enhances the expression of oligodendrocyte proteins following WML. (a to c) Immunoblots and representative graphs showing the protein expression of (a) mature oligodendrocyte marker, MBP, (b) OPC marker, PDGFαR and (c) pre-oligodendrocytes protein marker, CNPase by western blot. n = 3–5. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats. Values are mean ± SEM.

the groups treated with 0.1–100 μM ginkgolide B (Figure 7b to f). Overall, our data indicate that ginkgolide B could promote OPC differentiation.

Ginkgolide B attenuates the apoptosis

To confirm the effects of ginkgolide B after a WML, TUNEL staining was used to observe apoptosis. The data suggest that, compared to control SD rats, there are more apoptotic cells in the brain following WML (Figure 8b). However, the effect was reversed by ginkgolide B (Figure 8c to f). For the sake of making further efforts investigating the ameliorate influence of ginkgolide B on apoptosis at protein level, western blot was applied to assess protein level of cleaved caspase-3. Our data demonstrate that cleaved caspase-3 expression was upregulated following chronic cerebral hypoperfusion, and was decreased after ginkgolide B treatment (Figure 8g and h).

Ginkgolide B enhances AKT activities

Previous experiments suggest that the oligodendroglial development and apoptosis is essential through Akt signaling pathway.[38–40] To investigate the endogenous

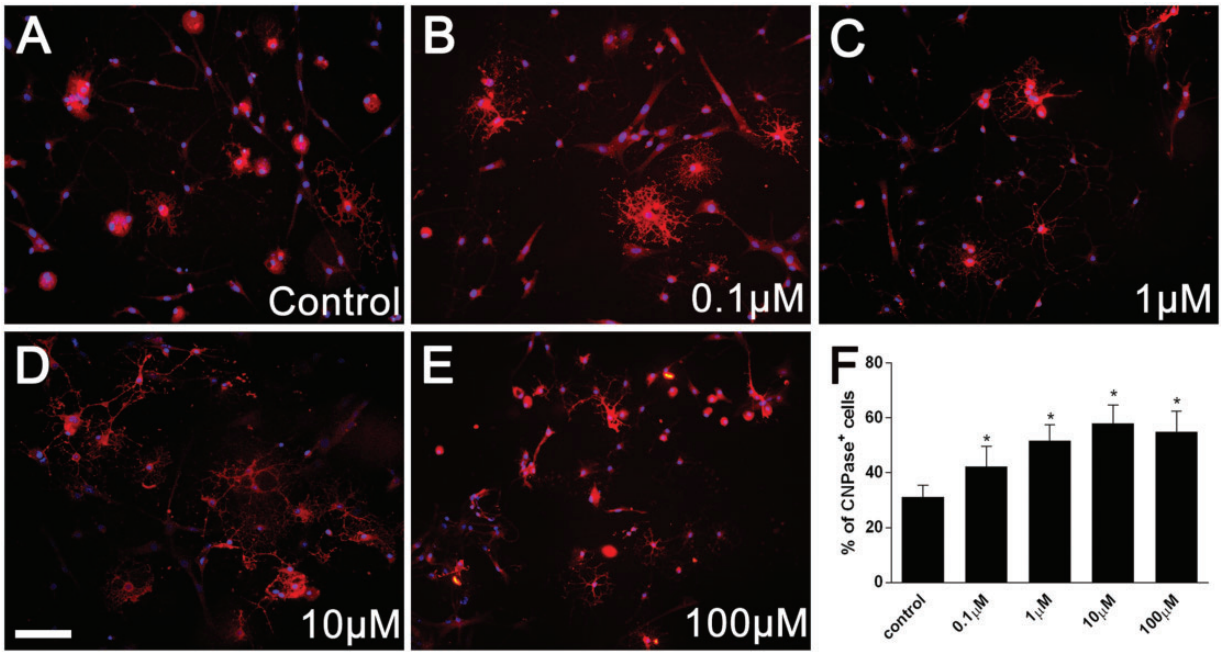

Figure 7. Ginkgolide B promotes cultured OPC differentiation in vitro. (a to e) showed immunofluorescence staining of OPCs cultured with (a) ethanol (control) and (b to e) 0.1–100 μM ginkgolide B. The cultured OPCs were single-stained with CNPase (red; a to e), and Hoechst 33342 (blue; a to e) was used to contrast the nuclei. (f) Data showed the percentage of $CNPase^+$ cells to all cells. *$p < 0.05$ vs. control group. Scale bars = 100 μm.

mechanism of ginkgolide B, we assessed the protein levels of phosphorylated-Akt, phosphorylated-CREB, and Bcl-2 after WML. Our results showed that, when compared with controls, 5 mg/kg ginkgolide B significantly increased MBP protein levels (Figure 9g and h) and markedly enhanced the Akt signaling pathway activity by upregulating phospho-Akt, phospho-CREB, and Bcl-2 expression (Figure 9a and c). In sharp contrast to these observations, the effect was reversed by treatment with the Akt inhibitor LY294002 (Figure 9a to h). In addition, ginkgolide B administration reduced the protein level of cleaved caspase-3 in the corpus callosum after chronic hypoperfusion, when compared to the control (Figure 9e). However, the effect was reversed by LY294002 administration (Figure 9f). Overall, our results demonstrate that ginkgolide B may promote oligodendrocyte differentiation and prevent apoptosis by enhancing Akt signaling pathway activity.

Discussion

Our data confirm firstly that ginkgolide B can improve learning and memory ability, alleviate myelin loss, reduce oligodendrocyte apoptosis, and promote the differentiation of OPC after WML. The therapeutic effect of ginkgolide B may be related to the enhancement of Akt phosphorylation.

Several studies have shown that late-life depressive disorder is always accompanied with vascular cognitive impairment.[41–43] Furthermore, it has been shown that ginkgolide B may alleviate the cognitive impairment of Alzheimer's disease.[44] Thus, to determine whether ginkgolide B has a therapeutic effect on learning and memory impairment but not depression after cerebral hypoperfusion, we conducted open field and elevated maze experiments. The data suggested that ginkgolide B excluded the effects on anxiety and depression in rats.

Oligodendrocyte, as myelinating sheaths, is vulnerable to ischemia by activating caspase pathways.[45–47] Oligodendrocyte apoptosis is involved in initiating demyelination.[48] Our findings indicate that ginkgolide B can alleviate oligodendrocyte apoptosis and myelin loss following WML. This suggested that the therapeutic result of ginkgolide B on myelin sheath may involve the reduction of oligodendrocyte apoptosis.

Moreover, our data demonstrated that ginkgolide B promotes OPC differentiation into oligodendrocytes and myelination in vivo and in vitro (Figures 2 to 4). We observed that ginkgolide B could upregulate the expression of markers in mature oligodendrocyte (such as APC and MBP) while suppressing those of OPC markers (such as PDGFαR). We induce that ginkgolide B could promote additional OPCs differentiation rather than proliferation. In addition, our data suggest that ginkgolide B has little effect on pre-mature oligodendrocytes (such as marker CNPase), which may indicate the balance of oligodendrocyte lineage.

Akt, as a serine/threonine kinase, is crucial to regulate cell development, growth, and survival. The Akt phosphorylation may have a beneficial effect on cell survival by preventing apoptosis. The phosphorylation of CREB by AKT leads to CREB transcription and activation,[49] which upregulates the protein expression level of Bcl-2.[50] The survival of oligodendrocytes after cerebral ischemia may be related to the phosphorylation of CREB.[51,52] Moreover, recent work has shown that ginkgolide B can activate the tyrosine phosphorylation of EGFR/SRC/FAK/Paxilin, which is related to the activation of PI3K.[53] The outcomes coincide with

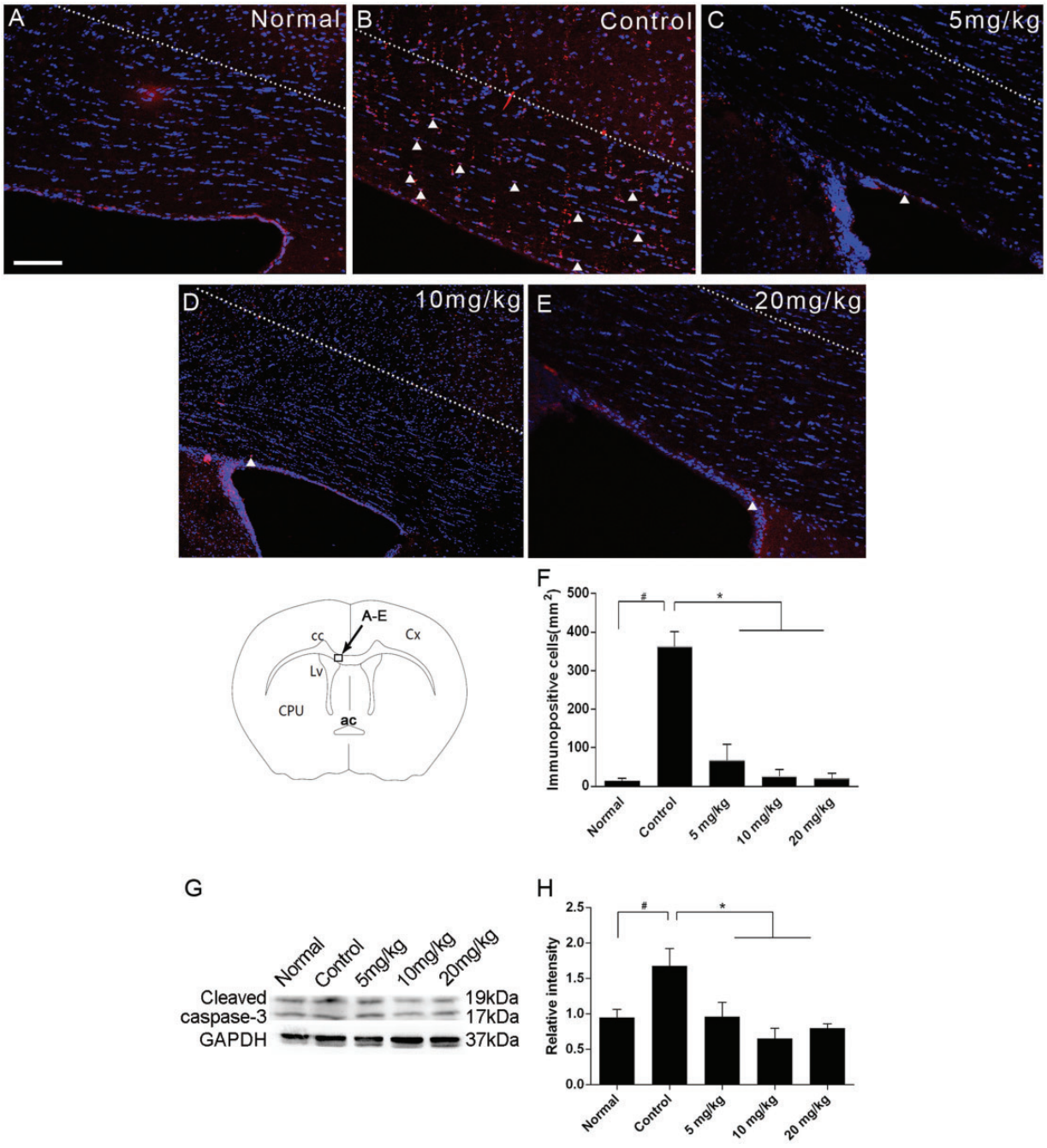

Figure 8. Ginkgolide B attenuates the apoptosis following WML. (a to e) showed the oligodendrocytes apoptosis of (a) normal, (b) control, and (c to e) 5–20 mg/kg ginkgolide B-treated rats by TUNEL staining. Data are the mean ± SEM of six sections each rat and five rats each group. (f) Quantitative analysis of TUNEL + cells (white triangle; a to e) per group. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats. Scale bars (a to e) = 50 μm. (g) Representative immunoblot image of cleaved caspase 3 of the normal, control, and 5–20 mg/kg ginkgolide B-treated rats. (h) Quantitative analysis of change in cleaved caspase 3 in each group. n = 3–5. #$p < 0.05$ vs. normal rats. *$p < 0.05$ vs. control rats. Data are mean ± SEM.

those from our experiments which showed that ginkgolide B treatment could upregulate Akt/CREB/Bcl-2 activity, suggesting that ginkgolide B induced Akt signaling pathway activation may involve oligodendrocyte survival. Furthermore, a line of evidence has shown that oligodendrocyte differentiation was associated with the Akt signaling pathway.[54–56] A group of studies have shown that ginkgolide B promotes neuron and astrocyte proliferation and differentiation via the Akt pathway.[25,26] Our study has shown that ginkgolide B treatment could enhance Akt activity, which suggests that ginkgolide B-mediated Akt activation may involve OPC differentiation. Together, the studies demonstrated that the Akt/CREB/Bcl-2 signaling pathway may have a central role in suppressing apoptosis and promoting OPC differentiation by ginkgolide B treatment.

All in all, the data demonstrate that ginkgolide B has a central role in promoting OPC differentiation and

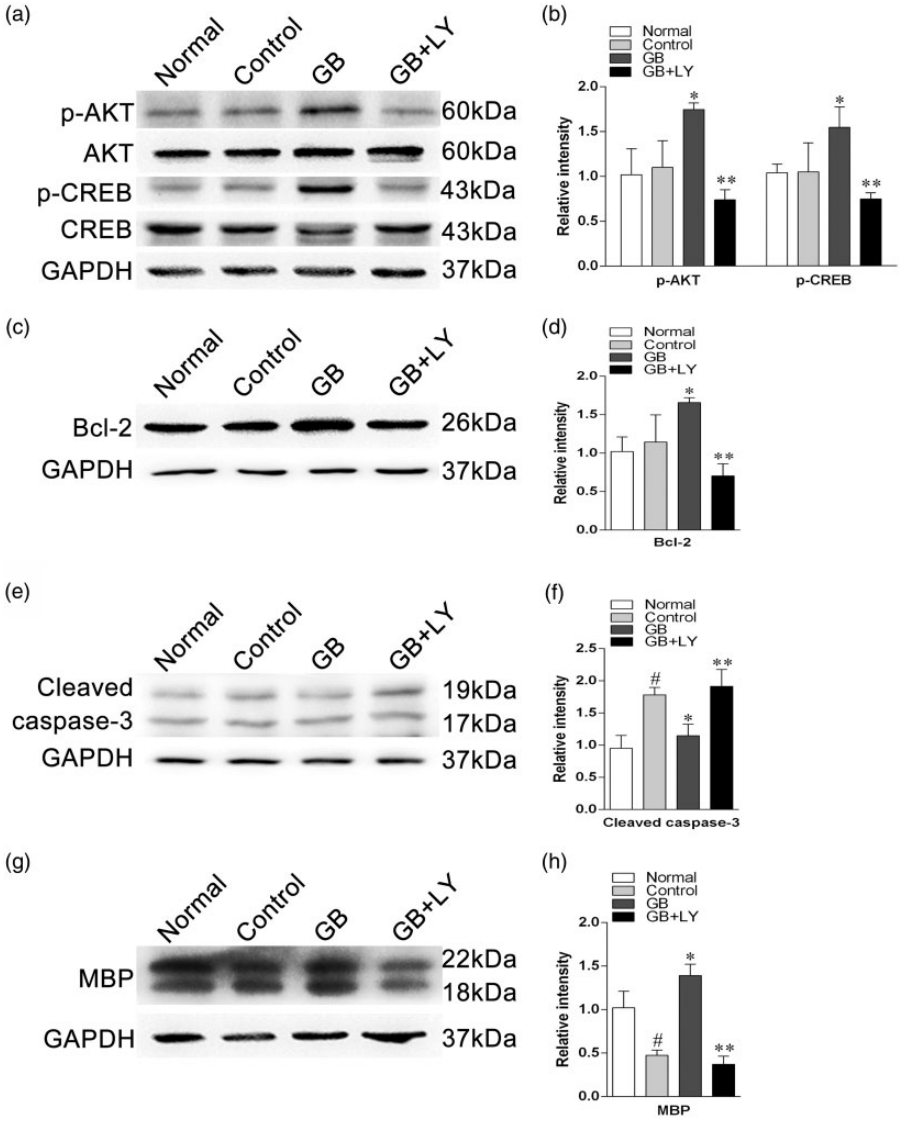

Figure 9. Ginkgolide B enhances MBP and decreases cleaved caspase 3 activities via AKT/CREB/Bcl-2 pathways after WML. (a to h) Representative western blot analysis shown in (a) phospho-AKT, phospho-CREB, (c) Bcl-2, (e) cleaved caspase 3, and (g) MBP protein expression of normal, control, 5 mg/kg ginkgolide B and 5 mg/kg ginkgolide B plus Akt inhibitor LY294002-treated rats. n = 3–5. #$p < 0.05$ vs. the normal. *$p < 0.05$ vs. control rats. **$p < 0.05$ vs. ginkgolide B treatment rats. Data are mean ± SEM.

oligodendrocyte survival following WML through activating Akt/CREB/Bcl-2 signaling pathway, which may potentially be a therapeutic agent for WML.

AUTHORS' CONTRIBUTIONS

JH and JY planned and executed the studies, data analysis, and drafted the manuscript. XJZ and HZ processed and analyzed the data. SLZ have made great efforts to revise the manuscript. GZ conceived the item. MS and ZRL facilitated design and analysis of the experiments. All the authors examined and agreed on the article.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was funded by National Natural Science Foundation of China (81471197 and 81070950).

ORCID iD

Zhirong Liu https://orcid.org/0000-0001-9661-6499

REFERENCES

1. Brinkmann BG, Agarwal A, Sereda MW, Garratt AN, Muller T, Wende H, Stassart RM, Nawaz S, Humml C, Velanac V, Radyushkin K, Goebbels S, Fischer TM, Franklin RJ, Lai C, Ehrenreich H, Birchmeier C, Schwab MH, Nave KA. Neuregulin 1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system. *Neuron* 2008;**59**:581–95

2. Raasch J, Zeller N, van Loo G, Merkler D, Mildner A, Erny D, Knobeloch KP, Bethea JR, Waisman A, Knust M, Del Turco D, Deller T, Blank T, Priller J, Bruck W, Pasparakis M, Prinz M. IkappaB kinase 2 determines oligodendrocyte loss by non-cell-autonomous activation of NF-kappaB in the central nervous system. *Brain* 2011;**134**:1184–98
3. Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke* 2003;**34**:1126–9
4. Akiguchi I, Tomimoto H, Wakita H, Kawamoto Y, Matsuo A, Ohnishi K, Watanabe T, Budka H. Topographical and cytopathological lesion analysis of the white matter in Binswanger's disease brains. *Acta Neuropathol* 2004;**107**:563–70
5. Targosz-Gajniak M, Siuda J, Ochudo S, Opala G. Cerebral white matter lesions in patients with dementia - from MCI to severe Alzheimer's disease. *J Neurol Sci* 2009;**283**:79–82
6. Farkas E, Luiten PG, Beri F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* 2007;**54**:162–80
7. Tullberg M, Fletcher E, DeCarli CM, Reed BR, Harvey DJ, Weiner MW, Chui HC, Jagust WJ. White matter lesions impair frontal lobe function regardless of their location. *Neurology* 2004;**63**:246–53
8. Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM. Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. *Brain* 2000;**123**:1845–9
9. Jalal FY, Yang Y, Thompson J, Lopez AC, Rosenberg GA. Myelin loss associated with neuroinflammation in hypertensive rats. *Stroke* 2012;**43**:1115–22
10. Simpson JE, Hosny O, Wharton SB, Heath PR, Holden H, Fernando MS, Matthews F, Forster G, O'Brien JT, Barber R, Kalaria RN, Brayne C, Shaw PJ, Lewis CE, Ince PG. Microarray RNA expression analysis of cerebral white matter lesions reveals changes in multiple functional pathways. *Stroke* 2009;**40**:369–75
11. Tomimoto H, Ihara M, Wakita H, Ohtani R, Lin JX, Akiguchi I, Kinoshita M, Shibasaki H. Chronic cerebral hypoperfusion induces white matter lesions and loss of oligodendroglia with DNA fragmentation in the rat. *Acta Neuropathol* 2003;**106**:527–34
12. de Lau LM, de Vries JM, van der Woude CJ, Kuipers EJ, Siepman DA, S, Smitt PA, Hintzen RQ. Acute CNS white matter lesions in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2009;**15**:576–80
13. Leroux P, Hennebert O, Legros H, Laudenbach V, Carmeliet P, Marret S. Role of tissue-plasminogen activator (t-PA) in a mouse model of neonatal white matter lesions: interaction with plasmin inhibitors and anti-inflammatory drugs. *Neuroscience* 2007;**146**:670–8
14. Wang LW, Tu YF, Huang CC, Ho C-J. JNK signaling is the shared pathway linking neuroinflammation, blood-brain barrier disruption, and oligodendroglial apoptosis in the white matter injury of the immature brain. *J Neuroinflammation* 2012;**17**:175
15. Medina-Rodríguez EM, Arenzana FJ, Pastor J, Redondo M, Palomo V, García de Sola R, Gil C, Martínez A, Bribián A, de Castro F. Inhibition of endogenous phosphodiesterase 7 promotes oligodendrocyte precursor differentiation and surviva. *Cell Mol Life Sci* 2013;**70**:3449–62
16. Mi S, Miller RH, Tang W, Lee X, Hu B, Wu W, Zhang Y, Shields CB, Zhang Y, Miklasz S, Shea D, Mason J, Franklin RJ, Ji B, Shao Z, Chédotal A, Bernard F, Roulois A, Xu J, Jung V, Pepinsky B. Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. *Ann Neurol* 2009;**65**:304–15
17. Van Strien ME, Baron W, Bakker EN, Bauer J, Bol JG, Brevé JJ, Binnekade R, Van Der Laarse WJ, Drukarch B, Van Dam A-M. Tissue transglutaminase activity is involved in the differentiation of oligodendrocyte precursor cells into myelin-forming oligodendrocytes during CNS remyelination. *Glia* 2011;**59**:1622–34
18. Kondo T, Raff M. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* 2000;**289**:1754–7
19. Levine JM, Reynolds R, Fawcett J. The oligodendrocyte precursor cell in health and disease. *Trends Neurosci* 2001;**24**:39–47
20. Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Baron-Van Evercooren A. Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. *Eur J Neurosci* 1999;**11**:4357–66
21. Liu X, Zhao G, Yan Y, Bao L, Chen B, Qi R. Ginkgolide B reduces atherogenesis and vascular inflammation in ApoE(–) mice. *PLoS ONE* 2012;**7**:e36237
22. Fang W, Deng Y, Li Y, Shang E, Fang F, Lv P, Bai L, Qi Y, Yan F, Mao L. Blood brain barrier permeability and therapeutic time window of Ginkgolide B in ischemia-reperfusion injury. *Eur J Pharm Sci* 2010;**39**:8–14
23. Lin Y, Wang R, Wang X, He RR, Wu YM. Effects of ginkgolide B on neuronal discharges in paraventricular nucleus of rat hypothalamic slices. *Neurosci Bull* 2008;**24**:345–50
24. Yu WH, Dong XQ, Hu YY, Huang M, Zhang ZY. Ginkgolide B reduces neuronal cell apoptosis in the traumatic rat brain: possible involvement of toll-like receptor 4 and nuclear factor kappa B pathway. *Phytother Res* 2012;**26**:1838–44
25. Tang Y, Huang B, Sun L, Peng X, Chen X, Zou X. Ginkgolide B promotes proliferation and functional activities of bone marrow-derived endothelial progenitor cells: involvement of Akt/eNOS and MAPK/p38 signaling pathways. *Eur Cell Mater* 2011;**21**:459–69
26. Wu X, Qian Z, Ke Y, Du F, Zhu L. Ginkgolide B preconditioning protects neurons against ischaemia-induced apoptosis. *J Cell Mol Med* 2009;**13**:4474–83
27. Cechetti F, Worm PV, Pereira LO, Siqueira IR, Netto CA. The modified 2VO ischemia protocol causes cognitive impairment similar to that induced by the standard method, but with a better survival rate. *Braz J Med Biol Res* 2010;**43**:1178–83
28. Jiwa NS, Garrard P, Hainsworth AH. Experimental models of vascular dementia and vascular cognitive impairment: a systematic review. *J Neurochem* 2010;**115**:814–28
29. Peng B, Guo QL, He ZJ, Ye Z, Yuan YJ, Wang N, Zhou J. Remote ischemic postconditioning protects the brain from global cerebral ischemia/reperfusion injury by up-regulating endothelial nitric oxide synthase through the PI3K/Akt pathway. *Brain Res* 2012;**1445**:92–102
30. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 1984;**11**:47–60
31. Carola VD, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behav Brain Res* 2002;**134**:49–57
32. Challa VR, Bell MA, Moody DM. Combined hematoxylin-eosin, alkaline phosphatase and high-resolution microradiographic study of lacunes. *Clin Neuropathol* 1990;**9**:196–204
33. Shaw K, MacKinnon MA, Raghupathi R, Saatman KE, Mclntosh TK, Graham DI. TUNEL-positive staining in white and grey matter after fatal head injury in man. *Clin Neuropathol* 2001;**20**:106–12
34. Itoyama Y, Sternberger NH, Webster HD, Quarles RH, Cohen SR, Richardson EP Jr. Immunocytochemical observations on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. *Ann Neurol* 1980;**7**:167–77
35. Hiremath MM, Saito Y, Knapp GW, Ting JP, Suzuki K, Matsushima GK. Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *J Neuroimmunol* 1998;**92**:38–49
36. Niu J, Wang L, Liu S, Li C, Kong J, Shen HY, Xiao L. An efficient and economical culture approach for the enrichment of purified oligodendrocyte progenitor cells. *J Neurosci Methods* 2012;**209**:241–9
37. Chen J, Zuo S, Wang J, Huang J, Zhang X, Liu Y, Zhang Y, Zhao J, Han J, Xiong L, Shi M, Liu Z. Aspirin promotes oligodendrocyte precursor cell proliferation and differentiation after white matter lesion. *Front Aging Neurosci* 2014;**6**:7
38. Kauffmann-Zeh A, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J, Evan G. Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* 1997;**385**:544–8
39. Kennedy SG, Wagner AJ, Conzen SD, Jordán J, Bellacosa A, Tsichlis PN, Hay N. The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev* 1997;**11**:701–13
40. Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature* 1999;**401**:86–90
41. Fujishima M, Maikusa N, Nakamura K, Nakatsuka M, Matsuda H, Meguro K. Mild cognitive impairment, poor episodic memory, and

late-life depression are associated with cerebral cortical thinning and increased white matter hyperintensities. *Front Aging Neurosci* 2014;**6**:306
42. Kling MA, Trojanowski JQ, Wolk DA, Lee VM, Arnold SE. Vascular disease and dementias: paradigm shifts to drive research in new directions. *Alzheimers Dement* 2013;**9**:76–92
43. Rosenberg PB, Mielke MM, Appleby BS, Oh ES, Geda YE, Lyketsos CG. The association of neuropsychiatric symptoms in MCI with incident dementia and Alzheimer disease. *Am J Geriatr Psychiatry* 2013;**21**:685–95
44. Bate C, Tayebi M, Williams A. Ginkgolides protect against amyloid-beta1-42-mediated synapse damage in vitro. *Mol Neurodegener* 2008;**3**:1
45. Li C, Guan T, Chen X, Li W, Cai Q, Niu J, Xiao L, Kong J. BNIP3 mediates pre-myelinating oligodendrocyte cell death in hypoxia and ischemia. *J Neurochem* 2013;**127**:426–33
46. Parthasarathy G, Philipp MT. The MEK/ERK pathway is the primary conduit for *Borrelia burgdorferi*-induced inflammation and P53-mediated apoptosis in oligodendrocytes. *Apoptosis* 2014;**19**:76–89
47. Stirling DP, Khodarahmi K, Liu J, McPhail LT, McBride CB, Steeves JD, Ramer MS, Tetzlaff W. Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J Neurosci* 2004;**24**:2182–90
48. Huang SQ, Tang CL, Sun SQ, Ang C, Xu J, Wang KJ, Lu WT, Huang J, Zhuo F, Qiu GP, Wu XY, Wei Q. Demyelination initiated by oligodendrocyte apoptosis through enhancing endoplasmic reticulum-mitochondria interactions and Id2 expression after compressed spinal cord injury in rats. *CNS Neurosci Ther* 2014;**20**:20–31
49. Salas TR, Reddy SA, Clifford JL, Davis RJ, Kikuchi A, Lippman SM, Menter DG. Alleviating the suppression of glycogen synthase kinase-3beta by Akt leads to the phosphorylation of cAMP-response element-binding protein and its transactivation in intact cell nuclei. *J Biol Chem* 2003;**278**:41338–46
50. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol* 2014;**15**:49–63
51. Sugiura S, Kitagawa K, Omura-Matsuoka E, Sasaki T, Tanaka S, Yagita Y, Matsushita K, Storm DR, Hori M. CRE-mediated gene transcription in the peri-infarct area after focal cerebral ischemia in mice. *J Neurosci Res* 2004;**75**:401–7
52. Tanaka K, Nogawa S, Ito D, Suzuki S, Dembo T, Kosakai A, Fukuuchi Y. Phosphorylation of cyclic adenosine monophosphate response element binding protein in oligodendrocytes in the corpus callosum after focal cerebral ischemia in the rat. *J Cereb Blood Flow Metab* 2001;**21**:1177–88
53. Aponte M, Jiang W, Lakkis M, Li MJ, Edwards D, Albitar L, Vitonis A, Mok SC, Cramer DW, Ye B. Activation of platelet-activating factor receptor and pleiotropic effects on tyrosine phospho-EGFR/Src/FAK/paxillin in ovarian cancer. *Cancer Res* 2008;**68**:5839–48
54. Cai Q, Yao Z, Li H. Catalpol promotes oligodendrocyte survival and oligodendrocyte progenitor differentiation via the Akt signaling pathway in rats with chronic cerebral hypoperfusion. *Brain Res* 2014;**1560**:27–35
55. Fischer R, Wajant H, Kontermann R, Pfizenmaier K, Maier O. Astrocyte-specific activation of TNFR2 promotes oligodendrocyte maturation by secretion of leukemia inhibitory factor. *Glia* 2014;**62**:272–83
56. Gomez O, Sanchez-Rodriguez A, Le M, Sanchez-Caro C, Molina-Holgado F, Molina-Holgado E. Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin (mTOR) pathways. *Br J Pharmacol* 2011;**163**:1520–32

(Received November 4, 2020, Accepted January 3, 2021)