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Green tea polyphenols inhibit cognitive impairment induced by chronic cerebral hypoperfusion via modulating oxidative stress

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Abstract

Responses to oxidative stress contribute to damage caused by chronic cerebral hypoperfusion, which is characteristic of certain neurodegenerative diseases. We used a rat model of chronic cerebral hypoperfusion to determine whether green tea polyphenols, which are potent antioxidants and free radical scavengers, can reduce vascular cognitive impairment and to investigate their underlying mechanisms of action. Different doses of green tea polyphenols were administered orally to model rats from 4 to 8 weeks after experimentally induced cerebral hypoperfusion, and spatial learning and memory were assessed using the Morris water maze. Following behavioral testing, oxygen free radical levels and antioxidative capability in the cortex and hippocampus were measured biochemically. The levels of lipid peroxidation and oxidative DNA damage were assessed by immunohistochemical staining for 4-hydroxynonenal and 8-hydroxy-2′-deoxyguanosine, respectively. Rats that received green tea polyphenols 400 mg/kg per day had better spatial learning and memory than saline-treated rats. Green tea polyphenols 400 mg/kg per day were found to scavenge oxygen free radicals, enhance antioxidant potential, decrease lipid peroxide production and reduce oxidative DNA damage. However, green tea polyphenols 100 mg/kg per day had no significant effects, particularly in the cortex. This study suggests that green tea polyphenols 400 mg/kg per day improve spatial cognitive abilities following chronic cerebral hypoperfusion and that these effects may be related to the antioxidant effects of these compounds.

Keywords: Green tea polyphenols; Learning and memory; Chronic cerebral hypoperfusion; Free radicals; Antioxidation

1. Introduction

Alzheimer disease (AD) and vascular dementia (VD) are the two primary types of dementia. Alzheimer disease is classified as a neurodegenerative disease but also is a vascular disorder [1], especially in the early stages, when chronic cerebral hypoperfusion increases AD-associated cognitive decline. Microvascular degeneration and chronic cerebrovascular hypoperfusion are characteristic of AD [2–4]. Chronic cerebral hypoperfusion in AD often precede the neurodegenerative changes, which are not merely a consequence but rather a pathogenic factor. In VD, cerebrovascular insufficiency and ischemic injury are believed to cause the brain dysfunction that underlies the dementia [5]. Thus, if the pathologic damage induced by chronic cerebral hypoperfusion could be diminished, cognitive impairment may be lessened.

The persistent decrease in cerebral blood flow (CBF) correlates with the severity of memory disturbances [6]. Chronic cerebral hypoperfusion is a major contributor to cognitive decline and a critical determining factor for dementia. Permanent bilateral occlusion of the common carotid arteries (2-VO) of rats is a well-characterized model used to investigate the cognitive and histopathologic consequences of chronic cerebral hypoperfusion [7,8]. Oxidative stress, a condition of cellular prooxidant–antioxidant disturbance that favors the prooxidant state, plays an important role in the pathogenesis of numerous neurodegenerative diseases and participates in the cognitive decline in the early stages of these diseases [9]. Permanent global hypoperfusion can increase oxidation and reduces antioxidant abilities [10]. Therefore, blocking the oxidative stress response associated with chronic cerebral hypoperfusion may be important for managing dementia.

Green tea is an extremely popular drink in eastern countries, and green tea polyphenols are natural plant flavonoids found in the tea plant leaves. Polyphenol antioxidants are known as catechins. The major tea catechins include (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-EC gallate (ECG) and (−)-EGC gallate (EGCG). Epigallocatechin gallate is the most active antioxidant of the tea catechins and is responsible for the “green tea effect.” The antioxidant activity of green tea polyphenols has been well studied in vivo and in vitro [11,12]. Long-term administration of green tea polyphenols improves spatial learning ability by lowering lipid peroxides [13]. In humans, orally administered catechin is absorbed, metabolized and excreted within 24 h. By simply drinking green tea, polyphenols can cross the blood brain barrier and have neuroprotective effects [14]. The questions remains, can green tea polyphenols improve cognitive

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function during chronic cerebral hypoperfusion? Green tea polyphenols to rats at dose levels of 100 mg/kg per day was equivalent to oral 40 mg/day EGCG (100 ml/day green tea) by human, and 400 mg/kg per day of green tea polyphenols for rats was equivalent to 400 ml/day of green tea for human. Here, we analyzed the effect of green tea polyphenols on spatial learning and memory and the oxidative stress response after a 2-VO injury to determine whether green tea polyphenols could improve cognitive function. Possible mechanisms for inhibiting cognitive decline were also explored.

2. Materials and methods

2.1. Animals

Ten-week-old male Wistar rats (250–300 g, n=100, at the beginning of the experiment,) were used for the study. All animals were housed two to three per cage at a temperature of 23±1°C with a regular 12-h light–dark cycle and free access to water and food. The animals were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Animal Ethics Committee of the Medical School of Wuhan University approved their use.

2.2. Drugs and experimental design

Green tea polyphenols were purchased from the Zhejiang Orient Tea Development Company. The purity of the green tea polyphenols was 98%, the content of total catechins was 79.67%, EGCG was 45.28%, EGC was 9.5%, EC was 6.68%, ECG was 12.88% and caffeine was 0.5%. Rats were randomly divided into four groups: sham-operated animals administered with saline (sham-operated group), 2-VO animals administered with saline (2-VO+saline group), 2-VO animals administered with 100 mg/kg per day of green tea polyphenols (2-VO+GTP100 group), 2-VO animals administered with 400 mg/kg per day of green tea polyphenols (2-VO+GTP400 group). After surgery, all rats were cared in cage free to food and water for 4 weeks. Then, rats were administered with green tea polyphenols dissolved in saline solution or an equal volume of normal saline. The green tea polyphenols or normal saline were administered orally by gastric intubation once daily for four consecutive weeks. The dosage volume for all groups was 10 ml/kg. Morris water maze was conducted after administration for 4 weeks. After the completion of the behavioral testing, the brain tissue was removed.

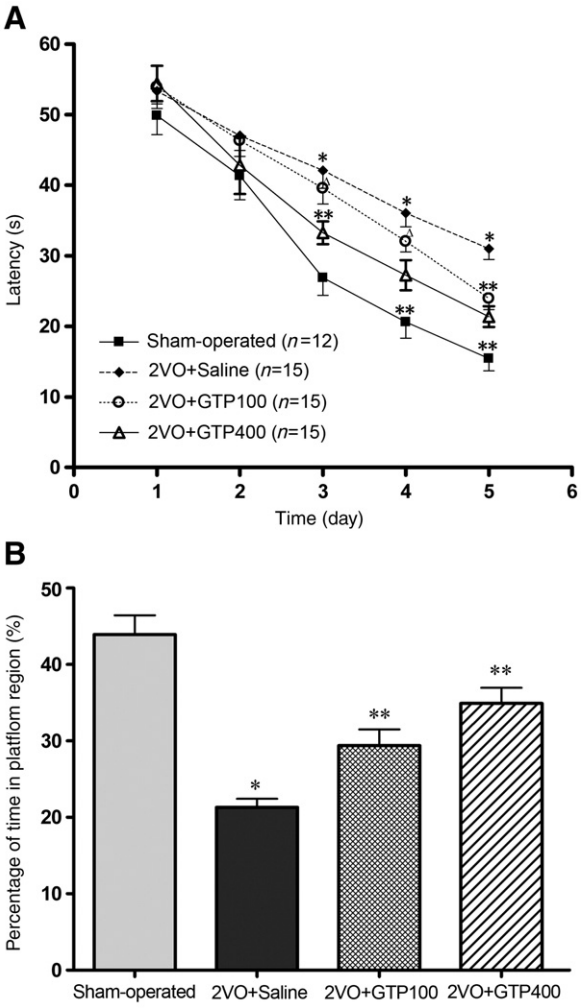

Fig. 1. Effect of green tea polyphenols on chronic cerebral hypoperfusion-induced deficits in spatial learning and memory as measured by the Morris water maze. (A) Change in the daily escape latency with time. (B) The percentage of time in the platform region in the probe trial without the platform (Day 6). All values are mean±S.E.M. *P<.01 vs. sham-operated rats; **P<.01 vs. 2-VO+saline rats; $^{\Delta}P$>.05 vs. 2-VO+saline rats.

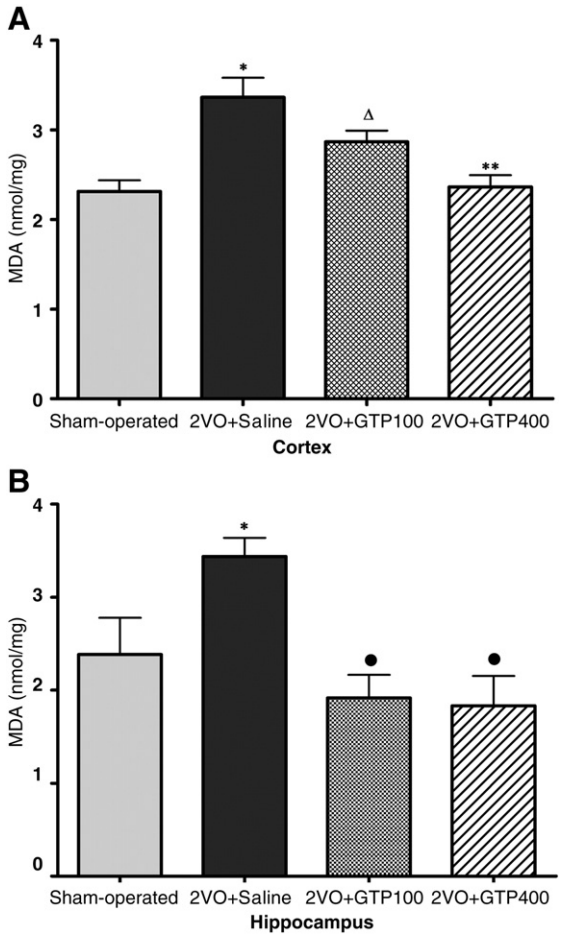

Fig. 2. Effect of green tea polyphenols on MDA levels in the cortex (A) and hippocampus (B) of rats with chronic cerebral hypoperfusion. Each bar point represents the mean±S.E.M. Sham-operated group, n=7; 2-VO+saline group, n=8; 2-VO+GTP100 group, n=8; 2-VO+GTP400 group, n=8. *P<.05 vs. sham-operated rats; **P<.05 vs. 2-VO+saline rats; $^{\Delta}P$>.05 vs. 2-VO+saline rats; $^{\bullet}P$<.01 vs. 2-VO+saline rats.

2.3. Surgery

Surgery to induced chronic cerebral hypoperfusion was carried out as described [15]. Food and water were withheld for 1 day prior to surgery. Rats were anesthetized with 10% chloral hydrate (350 mg/kg ip), and common carotid arteries were exposed bilaterally and carefully separated. In sequence, the bilateral common carotid arteries were double ligated with silk sutures. Sham-operated animals were treated in a similar manner, except that the common carotid arteries were not occluded. During the surgery, body temperature was maintained at 37.5±0.5°C, then rats were placed on a homeothermic tapetum until they recovered from anesthesia.

2.4. Morris water maze task

Spatial learning and memory were evaluated using Morris water maze. The rats whose visual system were compromised by 2-VO were excluded from the test. Eventually, there were 57 rats (sham-operated group: n=12; 2-VO+saline group: n=15; 2-VO+GTP100 group: n=15; 2-VO+GTP400 group: n=15) that underwent

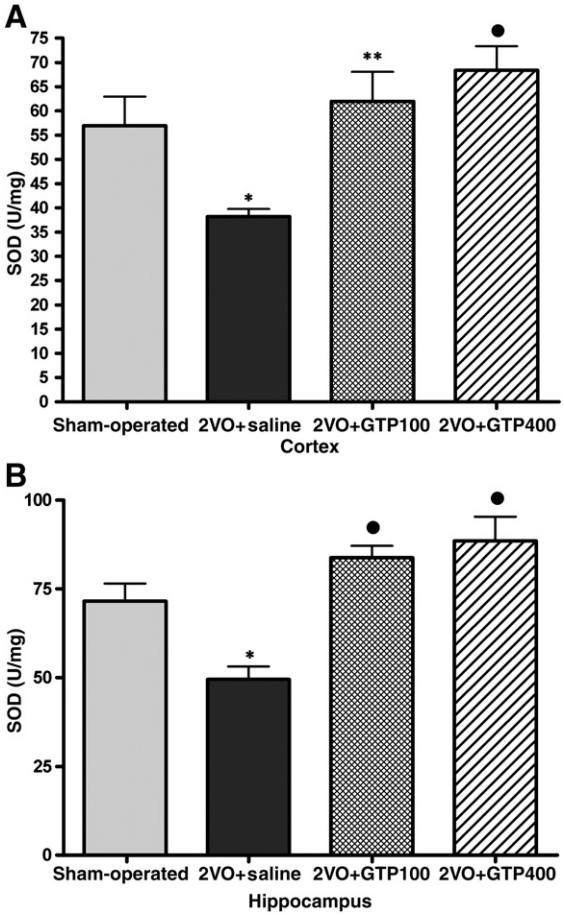

Fig. 3. Effect of green tea polyphenols on SOD levels in the cortex (A) and hippocampus (B) of rats with chronic cerebral hypoperfusion. Each bar represents the mean±S.E.M. Sham-operated group, n=7; 2-VO+saline group, n=8; 2-VO+GTP100 group, n=8; 2-VO+GTP400 group, n=8. *P<.05 vs. sham-operated rats; **P<.05 vs. 2-VO+saline rats; ●P<.01 vs. 2-VO+saline rats.

water maze. The swimming pool was a circular water tank, 120 cm in diameter, 60 cm high and 32 cm depth of water. A platform, 9 cm in diameter and 30 cm high, was placed inside the tank and was invisible from the water. The temperature of the swimming pool water was maintained at 24±2°C. The walls of the pool, the water and the platform were dyed black to conceal the platform. There were a number of orientation cues surrounding the pool to aid the rats learning the location of the platform. Each rat received four trials each day for five consecutive days. Rats were randomly put into the pool from each quadrant, facing the wall of the pool. The time to find the hidden platform was recorded if it was below 60 s. If the time exceeded 60s, the latency time was recorded as 60 s. Each rat was placed on the platform for 20 s regardless of whether it found the platform. On the sixth day, each rat was subjected to 60-s probe trials without the platform, and the time spent in the target quadrant where the platform had been set during training was recorded.

2.5. Biochemical analysis

After rats were sacrificed, brain cortex and hippocampus were removed on ice, blotted dry and weighed and then, afterward, homogenized with ice-cold saline to yield a 1% (w/v) homogenate. The supernatant was used to spectrophotometrically determine the level of malondialdehyde (MDA), the activity of superoxide dismutase (SOD) and total antioxidative capability (TAC) according to the procedure provided with the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China). Malondialdehyde, the degradation production of preoxidation lipid, reacts with thiobarbituric acid to form a pink chromogen that can be assayed by the method of Buege and Aust [16]. Malondialdehyde level reflects the degree of cell destruction by free radicals. Oxidation of hydroxylamine by the superoxide anion (O_2^-) from xanthine–xanthine oxidase forms nitrite, which reacts with a color-developing reagent to form a purple compound. Superoxide dismutase can neutralize the superoxide anion and lower the levels of nitrite. Thus, SOD activity reflects the capability of cleaning free radicals. Total antioxidative capability reflects the overall endogenous cellular antioxidative capability. These antioxidants can convert Fe^{3+} to Fe^{2+}, and the latter combines with phenanthroline to form colored and stable chelates.

2.6. Histopathology and immunohistochemistry

Rats were sacrificed after behavioral testing and perfusion-fixed for immunohistochemistry as described [17]. Rats were deeply anesthetized with 10% chloral hydrate, placed in a supine position, and the thorax was opened through a bilateral incision. A catheter was inserted into the left ventricle, and heparinized saline was infused until the perfusate from the right atrium was bloodless. Then, 4% paraformaldehyde (Sigma) in 300-ml phosphate-buffered saline (PBS) was infused. After perfusion fixation, the brain was removed and stored in fixative for at least 24 h. The brain was then embedded in paraffin wax and sectioned at multiple levels at 5 μm. Sections of parietal cortex and hippocampal CA1 area were stained with hematoxylin–eosin and immunohistochemically stained for 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxynonenal (4-HNE) to identify DNA damage and lipid peroxidation, respectively. The slides were placed in 0.3% H_2O_2 in PBS with 0.1% sodium azide for 11 min at room temperature to quench endogenous peroxidase activity. After blocking nonspecific reactions with bovine serum albumin, the slides were incubated with rabbit monoclonal anti-4-HNE (1:500; Chemicon) or goat monoclonal anti-8-OHdG (1:200; Chemicon) overnight at 4°C. The slides were washed with PBS twice for 20 min and incubated with diluted biotinylated secondary antibody for 1 h at room temperature. Slides were then washed again with PBS three times for 5 min and incubated with peroxidase-labeled streptavidin detection complex at 25°C for 10 min. The reaction product was developed by immersing the slides in diaminobenzidine solution that was prewarmed to 23°C. Slides were removed from water, and chromogenic enhancement was performed by placing the slides in 0.5% copper sulfate in PBS with Tween for 3 min at 25°C with gentle orbital rotation, followed by distilled water rinsing. In each study, a set of sections was stained in a similar way without the primary antibody as a negative control. The positive cells of parietal cortex and hippocampal CA1 area were counted at 400× magnification using five visual field from each animal by the biomedical image analysis system.

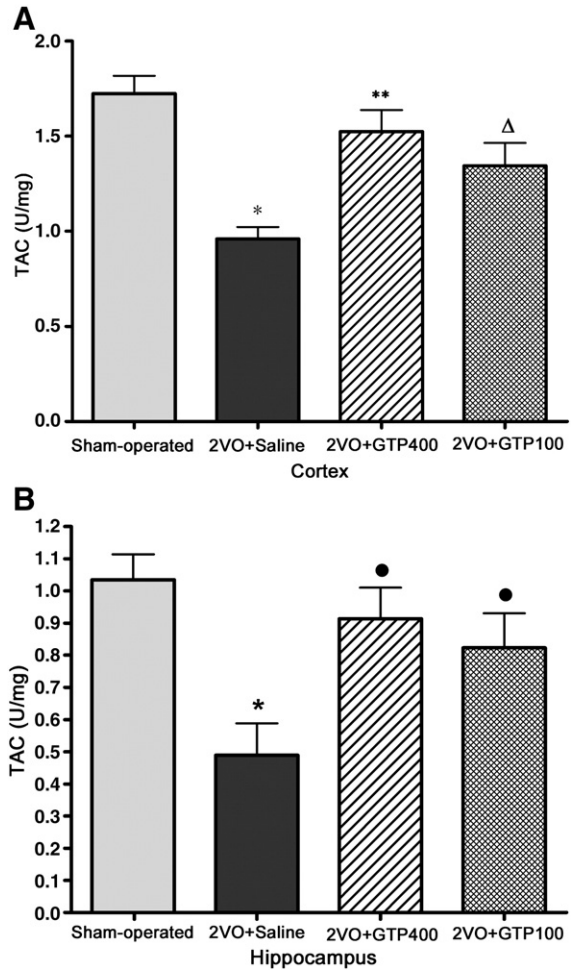

Fig. 4. Effect of green tea polyphenols on the TAC in the cortex (A) and hippocampus (B) of rats with chronic cerebral hypoperfusion. Each data point represents the mean±S.E.M. Sham-operated group, n=7; 2-VO+saline group, n=8; 2-VO+GTP100 group, n=8; 2-VO+GTP400 group, n=8. *P<.05 vs. sham-operated rats; **P<.05 vs. 2-VO+saline rats; ●P<.01 vs. 2-VO+saline rats; $^{\Delta}P$>.05 vs. 2-VO+saline rats.

2.7. Statistical analysis

All results are presented as mean±S.E.M. Differences in the escape latency in the Morris water maze were analyzed statistically with two-way analysis of variance followed by the Bonferroni test using GraphPad Prism 4.0 software. The other data were analyzed by one-way analysis of variance followed by the Tukey test using GraphPad Prism 4.0. Statistical significance was defined as $P<.05$.

3. Results

3.1. Green tea polyphenols improve spatial learning and memory deficits induced by chronic cerebral hypoperfusion

Rats were subjected to 5 days of trials in the Morris water maze to investigate spatial learning ability after 4 weeks of green tea polyphenols or normal saline. On the first two days of the trial, the escape latency did not differ significantly between groups ($P>.05$). Beginning on Day 3, rats in the chronic cerebral hypoperfusion group took longer to find the platform than the sham-operated rats ($P<.01$). The escape latencies of hypoperfusion rats treated with 100 mg/kg per day of green tea polyphenols were shorter, but not significantly different, than that of saline-treated rats ($P>.05$) on Days 3 and 4. The 400 mg/kg per day of green tea polyphenol-treated hypoperfusion rats spent significantly less time finding the platform than the saline-treated hypoperfusion rats ($P<.05$) beginning on Day 3 (Fig. 1A). In the probe trials, shown in Fig. 1B, memory was evaluated by measuring the percentage of time in the target quadrant without the platform. The chronic cerebral hypoperfusion rats stayed in the platform region less time than the sham-operated rats ($P<.01$). Administration of 400 or 100 mg/kg per day of green tea polyphenols significantly increased the amount of time of hypoperfusion rats spent in the platform region relative to the saline group of hypoperfusion rats ($P<.01$).

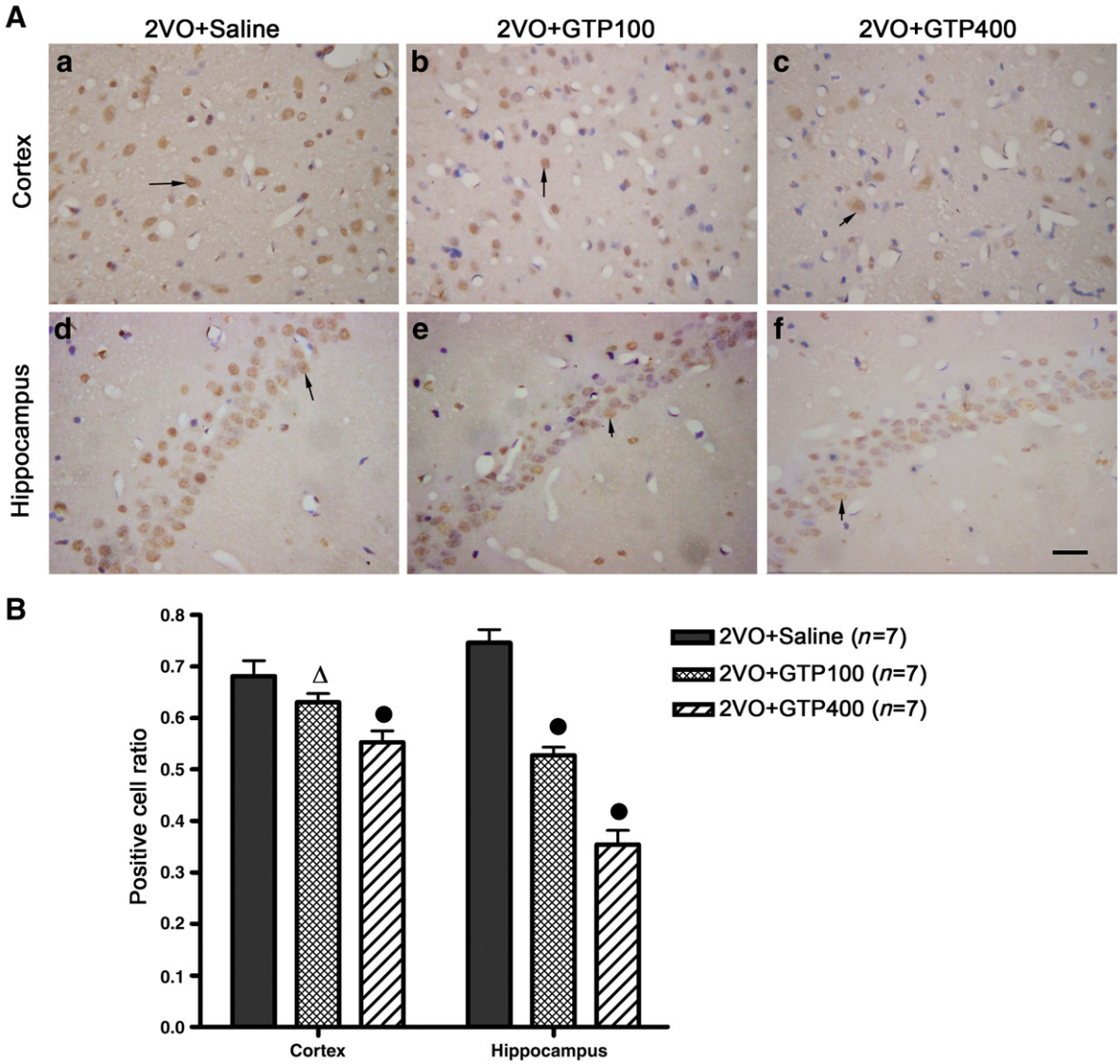

Fig. 5. Effect of green tea polyphenols on lipid peroxidation induced by chronic cerebral hypoperfusion. (A) Representative photomicrographs of 4-HNE immunohistochemistry in the cortex and hippocampus after chronic cerebral hypoperfusion and treatment. "a and d" show the 4-HNE-positive cells in the cortex and hippocampus, respectively, after chronic cerebral hypoperfusion; "b and e" show the effect of 100 mg/kg per day of green tea polyphenols in the cortex and hippocampus, respectively; "c and f" show the effect of 400 mg/kg per day of green tea polyphenols in the cortex and hippocampus, respectively. Scale bar=20 μm. Magnification ×400. (B) The proportion of 4-HNE-positive cells to total cells in the cortex and hippocampus is decreased by 400 mg/kg per day of green tea polyphenols. Each bar represents the mean±S.E.M. Sham-operated group, $n=5$; 2-VO+saline group, $n=7$; 2-VO+GTP100 group, $n=7$; 2-VO+GTP400 group, $n=7$. $^{●}P<.01$ vs. 2-VO+saline rats; $^{Δ}P>.05$ vs. 2-VO+saline rats.

3.2. Green tea polyphenols inhibit the chronic cerebral hypoperfusion-induced oxidative stress response

After testing cognitive function, the cortex and hippocampus of rats were separated to measure the levels of oxygen free radicals and antioxidant capability. Malondialdehyde levels in the cortex and hippocampus of 2-VO rats were greater than in sham-operated rats (Fig. 2A and B). Superoxide dismutase activity and TAC were reduced after chronic cerebral hypoperfusion relative to the sham-operated group (Figs. 3 and 4), demonstrating that chronic cerebral hypoperfusion can induce oxidative injury. Administration of green tea polyphenols decreased MDA levels and increased SOD activity and TAC. Green tea polyphenols 400 mg/kg per day significantly reduced the oxidative stress response in the cortex relative to the saline group ($P<.05$), whereas the group treated with 100 mg/kg per day of green tea polyphenols did not differ significantly from the saline group ($P>.05$) (Figs. 2A, 3A, 4A). Green tea polyphenols 100 and 400 mg/kg per day significantly decreased MDA levels and enhanced SOD activity and TAC in the hippocampus relative to the saline group ($P>.05$) (Figs. 2B, 3B, 4B).

3.3. The effect of green tea polyphenols on DNA damage and lipid peroxidation

Immunohistochemical analysis of 8-OHdG and 4-HNE reflects the extent of oxidative DNA damage and lipid peroxidation, respectively [17,18]. After chronic cerebral hypoperfusion, there were a number of 4-HNE- and 8-OHdG-positive cells in the cortex and hippocampus (Figs. 5 and 6). Positive staining for 4-HNE is seen in neuronal perikarya and axons, whereas 8-OHdG staining predominantly occurs in nuclei and, to a lesser extent, in the cytoplasm [17]. Green tea polyphenols 400 mg/kg per day decreased the number of 4-HNE- and

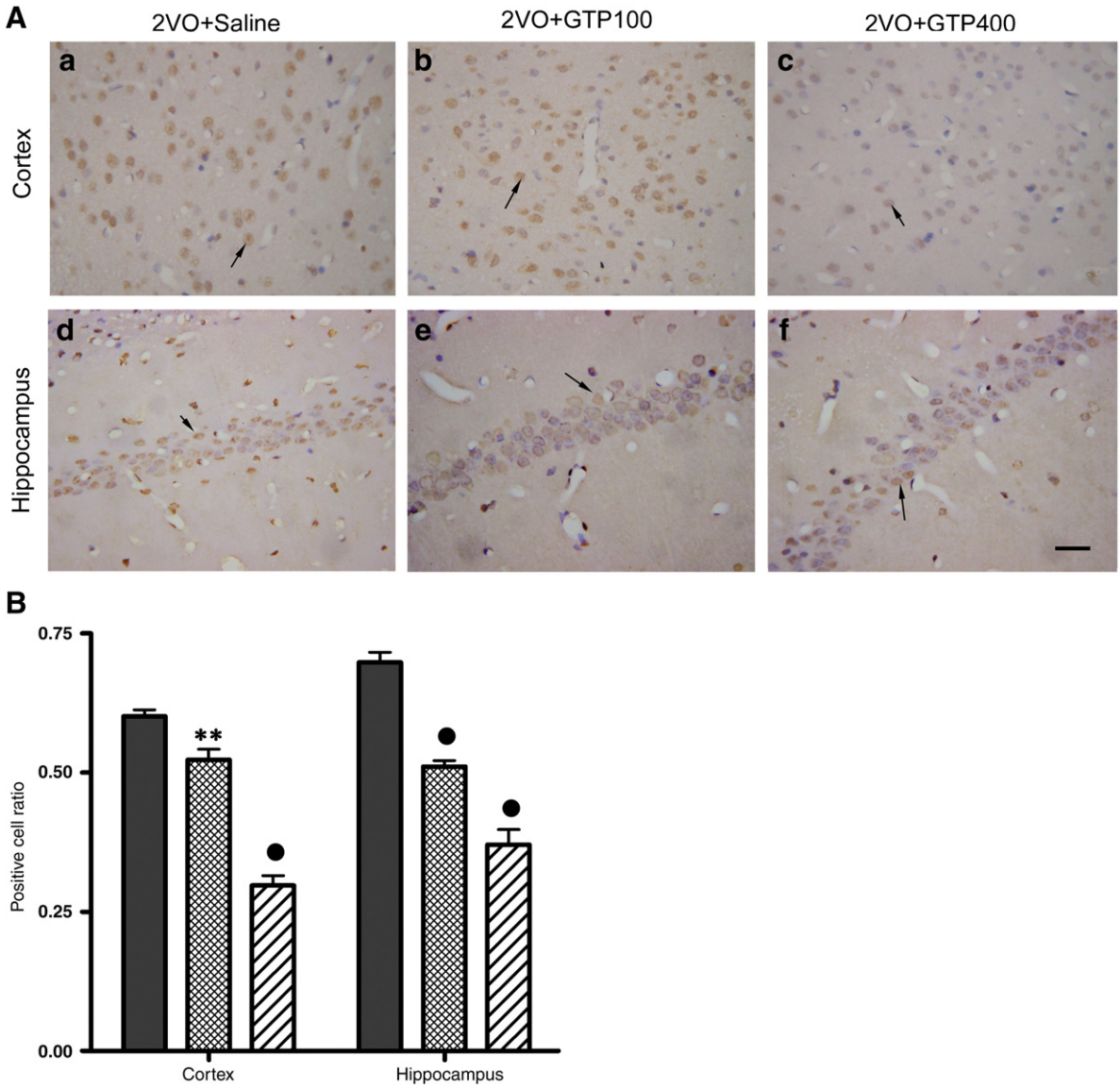

Fig. 6. Effect of green tea polyphenols on oxidative DNA damage induced by chronic cerebral hypoperfusion. (A) Representative photomicrographs of 8-OHdG immunohistochemistry in the cortex and hippocampus after chronic cerebral hypoperfusion and treatment. "a and d" show the 8-OHdG-positive cells in the cortex and hippocampus after chronic cerebral hypoperfusion; "b and e" show that 100 mg/kg per day of green tea polyphenols decrease the number of 8-OHdG-positive cells in the cortex and hippocampus, respectively; "c and f" show the effect of 400 mg/kg per day of green tea polyphenols on 8-OHdG-positive cells in the cortex and hippocampus, respectively. Scale bar=20 μm. Magnification ×400. (B) The proportion of 8-OHdG-positive cells to total cells in the cortex and hippocampus is decreased by 400 mg/kg per day of green tea polyphenols. Each bar represents the mean±S.E.M. Sham-operated group, $n=5$; 2-VO+saline group, $n=7$; 2-VO+GTP100 group, $n=7$; 2-VO+GTP400 group, $n=7$. $^{\bullet}P<.01$ vs. 2-VO+saline rats; $^{**}P<.05$ vs. 2-VO+saline rats.

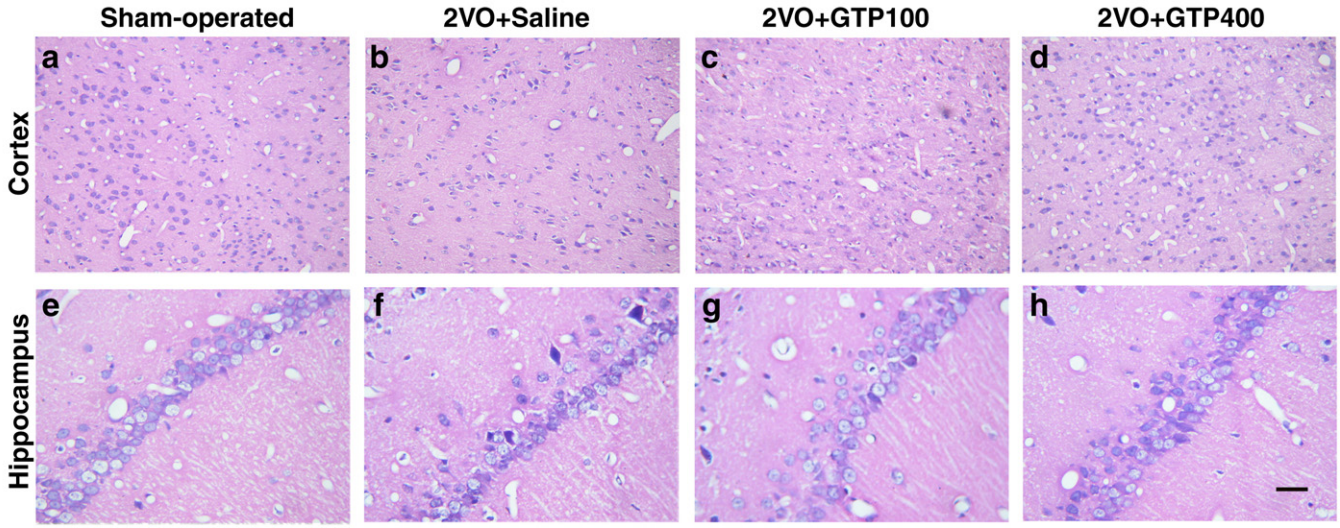

Fig. 7. Effect of green tea polyphenols on morphological changes induced by chronic cerebral hypoperfusion in parietal cortex and hippocampal CA1 area. (a and e) sham-operated group; (b and f) 2-VO+saline group; (c and g) 2-VO+GTP100 group; (d and h) 2-VO+GTP400 group. Magnification ×400.

8-OHdG-stained cells in the cortex and hippocampus. Green tea polyphenols 100 mg/kg per day reduced the number of 4-HNE- and 8-OHdG-positive cells in the hippocampus but did not significantly affect lipid peroxidation in the cortex (Figs. 5 and 6).

3.4. The effect of green tea polyphenols on morphological changes

Hematoxylin–eosin staining was used to survey the morphological changes after chronic cerebral hypoperfusion and treatment. In the cortex and hippocampus, marked morphological changes were visualized in hypoperfusion group: neuronal cell loss, glial proliferation, nuclei shrinkage and dark staining of neurons. Green tea polyphenols (400 mg/kg per day) markedly reduced the morphological changes (Fig. 7).

4. Discussion

The present study demonstrates that green tea polyphenols improved cognitive deficits induced by chronic cerebral hypoperfusion in rats. This neuroprotective effect may be due to the free radical scavenging and antioxidative properties of green tea polyphenols.

Permanent bilateral occlusion of the common carotid arteries of rats can reproduce the condition of chronic cerebral hypoperfusion and is regarded as a suitable model for studying the pathophysiology of learning and memory deficits associated with cerebral circulation impairments [8,10]. In the acute phase immediately after the start of bilateral occlusion of the common carotid arteries, the CBF drops sharply and reduces to 33% to 45% of the control in the cortex and to 60% in the hippocampus [19]. However, at 1 week, the CBF values starts to gradually recover. After 4 weeks of 2-VO, the CBF decreases to 69.8% in the cortex and to 66.3% in the hippocampus; the condition closely resembles the reduced CBF that occurs with human aging and dementia [8]. We investigated the cognitive impairment and pathophysiological changes that occurred after 4 weeks of occlusion and then administered green tea polyphenols for 4 weeks to explore the effects of green tea polyphenols on chronic cerebral hypoperfusion.

Our results showed that spatial learning and memory are impaired in rats after chronic cerebral hypoperfusion, consistent with previous reports [8,20–22]. Learning and memory in rats with chronic cerebral hypoperfusion were gradually impaired as the duration of ischemia increased [15]. The permanent neuronal damage induced by 2-VO can result in memory failure [8]. When rats in the chronic cerebral hypoperfusion group were administered green tea polyphenols, cognitive function and morphological changes improved. Spatial learning and memory were enhanced by two doses of green tea polyphenols, but only 400 mg/kg per day of green tea polyphenols had a significant effect, suggesting that 400 mg/kg per day of green tea polyphenols can ameliorate cognitive impairment in 2-VO rats.

Epidemiological research shows that hearty consumption of green tea is associated with a lower prevalence of cognitive impairment; the odds ratios for the cognitive impairment associated with different frequencies of green tea consumption are 1.00 for ≤3 cups/week, 0.62 for 4 to 6 cups/week or 1 cup/day and 0.46 for ≥2 cups/day (1 cup=100 ml) [23]. When rats are infused with Abeta (1–40) into the cerebral ventricle, the number of working memory errors increased. Green tea catechins decrease the Abeta-induced increase in the number of working memory errors and reduce the level of hippocampal lipid peroxide and cortico-hippocampal reactive oxygen species (ROS) [24]. The hippocampal CA1 area and cortex are the key structures for cognitive function, and thus, the decline of spatial learning and memory may be induced by deterioration of either the hippocampus or the cortex. To explore the neuroprotective mechanisms underlying the effects of green tea polyphenols, we studied the effects of green tea polyphenols on oxidative stress in the hippocampus and cortex during chronic cerebral hypoperfusion.

Permanent cerebral hypoperfusion produces excess free radicals [10,25] and ROS that can damage the weakened antioxidant defense system of the brain, thereby inducing neuronal degeneration and death. Our study showed that oxygen free radicals emerged and antioxidant abilities were compromised after chronic cerebral hypoperfusion, which can lead to damage of cellular proteins, lipids and DNA. After chronic cerebral hypoperfusion, antioxidant capabilities changed in the cortex and hippocampus. Liu et al [10] found elevated SOD activity in the cortex and hippocampus of permanent 2-VO model animals and considered this a compensatory rise in antioxidant activity that indicated the brain's antioxidant machinery was activated when overwhelmed by oxidative stress. There was also evidence for reduced antioxidant activity after chronic cerebral

hypoperfusion [26]. Our results indicate that the antioxidant machinery was damaged after 2-VO.

Malondialdehyde is produced by free radical-catalyzed peroxidation of unsaturated fatty acids in cell membranes, and SOD and TAC play important roles in injury brought about by the antioxidative response [26]. 4-HNE, a lipid peroxidation and cytotoxic byproduct, has direct toxic effects on neurons and oligodendrocytes after cerebral ischemia [27], and 4-HNE-modified protein can be used as a marker for oxidative neuronal damage [20]. The formation and accumulation of 8-OHdG, a hallmark of oxidative DNA damage caused by direct attacks by hydroxyl radicals, may damage active genes in corresponding cells [28]. In our present study, green tea polyphenols (400 mg/kg per day) lessened free radical damage to cells, enhanced antioxidant abilities and reduced lipid peroxidation products and oxidative DNA damage after chronic cerebral hypoperfusion, in both the cortex and hippocampus. Green tea polyphenols (100 mg/kg/d) had more modest effects, the oxidative stress response in the hippocampus was lessened, but there was no significant effect in the cortex. Hippocampal damage appeared beginning 1 week after the initiation of 2-VO and gradually extended to the cortex during a 4-week period [29]. Therefore, the effect of 100 mg/kg per day of green tea polyphenols on the hippocampus might reflect the fact that the hippocampus is damaged earlier. The neuroprotective effects of green tea polyphenols were linked to its multitargeted inhibition of oxidative stress. Unno et al. [30] found that green tea catechin intake can inhibit cognitive dysfunction and prevent oxidative DNA damage in mice with accelerated senescence. EGCG, a major polyphenol present in green tea that prevents oxidative damage in brain, can augment the activities of antioxidant enzymes, like SOD, catalase and glutathione peroxidase, and nonenzymatic antioxidants, like tocopherol, in aged rat brain to alleviate age-associated oxidative damage [31]. Green tea polyphenols 400 mg/kg per day may up-regulate endogenous antioxidants such as SOD. On the basis of the scavenging effects of the superoxide anion and hydroxyl radicals, the rank order of antioxidant abilities of green tea components is EGCG>ECG>EGC>EC [32]. Antioxidant abilities have been attributed to the presence of the C-ring gallate group and the A ring [33].

Green tea polyphenols prevent oxidative damage to proteins by restoring protein carbonyl levels and antioxidant enzyme status [31]. Antioxidative enzymes are activated by green tea catechin intake, and the antioxidative potency increases with continued ingestion of green tea [13]. Green tea polyphenols have been reported to have potent radical scavenging abilities [32] and can chelate metals to prevent metal-catalyzed free radical formation [34]. Green tea polyphenols can inhibit ROS accumulation by inhibiting xanthine oxidase, which catabolizes purines to produce uric acid and ROS [12]. Increased ROS production can lead to oxidative damage of lipids and DNA and, ultimately, apoptotic cell death and cognitive impairment. Green tea polyphenols can protect DNA from •OH radical-induced strand breaks and base damage through fast chemical repair of DNA radicals [35].

In our present study, there existed some shortages in elucidating the effects of green tea polyphenols on cognitive function. For example, we need test a classic antioxidant to support our conclusion. In addition, green tea polyphenols have other multiple neuroprotective effects. Green tea polyphenols could inhibit activation of the NF-κB (nuclear factor κB), ERK (extracellular signal regulated kinase), and p38 MAP (mitogen-activated protein) kinase pathways through antioxidant mechanisms [36] and inhibit Abeta aggregation and Abeta fibrils/oligomers formation [37]. Green tea polyphenols also dramatically inhibit acetylcholinesterase activity [38]. These actions may help to explain how green tea polyphenols improve cognitive function. In future, we need more tests to explore the neuroprotective mechanism of green tea polyphenols after chronic cerebral hypoperfusion.

In conclusion, our study indicates that green tea polyphenols scavenge free radicals, enhance antioxidant capabilities and reduce lipid peroxidation and oxidative DNA damage after chronic cerebral hypoperfusion, which may underlie cognitive function improvement. The results suggest that green tea polyphenols might be effective for managing vascular cognitive impairment.

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