doi.org/10.36721/PJPS.2020.33.1.SP.469-479.1

Prevention of systemic inflammation and neuroprotective effects of Qingda granules against angiotensin II-mediated hypertension

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Abstract: Qingxuan Jiangya Decoction (QXJYD), prescribed by academician Ke-ji Chen, has long been used as a Traditional Chinese Medicine formula in blood pressure control and has achieved good clinical outcomes in hypertensive patients. Qingda granules (QDGs), which is a formula simplified from QXJYD, might serve as a novel anti-hypertensive pharmaceutical. However, the functional mechanism of QDGs remains unclear. This study aimed to evaluate the effect of QDGs against the elevation of blood pressure, systemic inflammation and brain injury in Ang II-mediated hypertensive mice. Ang II-mediated hypertensive mice were treated with 28.63mg QDG of per mouse every day. The blood pressure of all mice was measured on days 0, 1, 3, 5, 7, 14 and 28 by using the tail-cuff plethysmograph method. Following 28 days of treatment, the mice were sacrificed and their whole blood and brain tissues were used for analysis. The results showed that QDGs significantly decreased elevated systolic and diastolic blood pressure in Ang II-mediated hypertensive mice while body weight did not change, which demonstrated anti-hypertensive activities of QDGs without obvious toxicity. QDGs significantly attenuated the level of serum cytokines (IL-6, TNF-α) and chemokines (MCP-1, MIP-1α, RANTES) in the Ang II-mediated hypertensive mice. Moreover, pathological staining showed that QDGs significantly ameliorated cerebral histopathology changes, reduced the loss of neurons and activations of astrocytes. Additionally, QDGs inhibited neuronal apoptosis by down-regulation of Bax expression and up-regulation of Bcl-2 expression. These results suggested that QDGs exhibited excellent anti-hypertensive properties by preventing systemic inflammation and providing neuroprotective effects against Ang II-mediated hypertension.

Keywords: Qingda granules, hypertension, inflammation, brain injury, apoptosis.

INTRODUCTION

Cardiovascular disease (CVD) includes myocardial infarction, heart failure, stroke, and hypertension, and is one of the leading causes of death worldwide. Particularly, hypertension is the main trigger for CVD and the nearly 16.5% of annual deaths worldwide occur from hypertension-related causes (Organization, 2013). Hypertension is defined well as development of systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥90 mmHg (Tabassum and Ahmad, 2011). Every increase of 20/10 (SBP/DBP) implies advantage section of hypertension (Goodnow, 2007). The underlying mechanisms of hypertension are complex and its serious comorbidities are even more dangerous, such as cardiac hypertrophy, heart failure, exacerbation of coronary heart disease, stroke and hypertensive chronic kidney disease (Rubattu *et al.*, 2014). Therefore, prevention and treatment of elevated blood pressure (BP) will have a strong effect on the health status of human populations.

It has been proven that the renin-angiotensin-aldosterone system (RAAS) has a crucial role in development of hypertension. Activation of RAAS in response to a decrease in cardiac output contributes to secretion of renin, which converts angiotensinogen to angiotensin I (Ang I) (Dzau *et al.*, 1987). Angiotensin-converting enzyme 1 (ACE-1) catalyzes conversion of inactive Ang I to angiotensin II (Ang II) (Yee *et al.*, 2010). Ang II, a potent vasoconstrictive peptide hormone, stimulates secretion of aldosterone, which could enhance reabsorption of sodium and water in renal tubules and lead to excretion of potassium. This causes a rise in the intravascular fluid volume leading to an increase in BP (Parvanova *et al.*, 2005), which will be elevated if the RAAS is abnormally active. Presently, many medicines, including diuretics, β-blockers, ACE inhibitors, calcium channel antagonists, and angiotensin receptor blockers (ARBs), are used to block different steps in this system to

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reduce BP (Vercruysse *et al.*, 2005). However, high BP is managed in only 34% of patients and the hypertension-related comorbidities have not yet been effectively treated (Jie and Xiong, 2012). For instance, β-blockers have little effect on improving arterial structure, yet they decrease the levels of plasma renin and Ang II (Blumenfeld *et al*., 1999). Some studies have reported that BP lowering alone is insufficient to improve pial artery or middle cerebral artery structure by using RAAS-inhibition medicines (Chillon and Baumbach, 1999, Koller and Toth, 2012). Therefore, it is required to develop novel pharmaceuticals for prevention and treatment of hypertension.

In Traditional Chinese Medicine (TCM), the Qingxuan Jiangya Decoction (QXJYD) formula, prescribed by academician Ke-ji Chen, has long been used as a TCM formula in achieving BP control and good clinical outcomes in hypertensive patients. Qingda granules (QDGs), which is a simplified formulation of QXJYD, consists of 12g *Gastrodia*, 10 g *Uncaria rhynchophylla*, 6g *Scutellaria baicalensis,* and 5g *Nelumbo nucifera* Gaertn*.* Numerous studies have also shown that these compounds had anti-hypertensive activities. For example, acidic polysaccharides purified from *Gastrodia* reduced hypertension and improved serum lipid levels (Lee *et al.*, 2012), *U. rhynchophylla* total alkaloids exhibited properties of anti-hypertension and protected blood vessels attributed to relief of overall low-grade inflammation (Li *et al.*, 2015), and baicalin, a flavonoid compound extracted from the roots of *S. baicalensis*, has been shown to effectively attenuate pulmonary hypertension via inhibition of NF-κB signaling to further activate BMP signaling (Zhang *et al.*, 2017). Although a small number of references have hinted that *N. nucifera* Gaertn has a therapeutic effect on hypertension, it has been shown to have preventive and anti-inflammatory effects on inflamed macrophages induced by lipopolysaccharide (LPS) (Liao and Lin, 2012).

There are currently no reports on the effect and functional mechanisms of QDGs on treatment of hypertension. Mini-osmotic pumps have been used to infuse Ang II into mice to construct a model of hypertension that has been widely used to study the pathogenesis of hypertension and in anti-hypertensive drug screening because of its simple operation, high success rate, ease of copying, stability, and short building time. It is generally known that Ang II has a pivotal role in the development of hypertension and is associated with the key events in inflammation. Hypertension, a major risk factor for stroke, has damaging effects on the brain and its vessels (Pires *et al.*, 2013). Consequently, this study aimed to illustrate if QDGs could attenuate elevated SBP and DBP, ameliorate inflammation, and alleviate brain injury in Ang II-mediated hypertensive mice. It is hoped that the study results will serve as a theoretical basis for the widespread use of QDGs as a novel pharmaceutical in the prevention and treatment of hypertension.

MATERIALS AND METHODS

Materials and reagents

A Histostain-Plus Kit was obtained from Mai XinBiotech Co., Ltd. (Fuzhou, China). Bcl-2 and GFAP antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). NeuN and Bax antibodies were obtained from Abcam (Cambridge, MA, USA). Bio-Plex Pro™ Mouse Cytokine Grp I Panel 23-Plex kit was obtained from Bio-rad (Hercules, CA, USA). Mini-osmotic pump (model:2004) was provided from Alezt (Cupertino, CA, USA). All of the other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of QDGs

QDGs were prepared and provided from Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangsu, China) and was authenticated by Dr. Lin Shan (Academy of Integrative Medicine, Fujian University of Traditional Chinese Medicine). According to the animal's weight, the QDGs were dissolved in 0.9% NaCl to a concentration of 57.25mg/mL and stored at -20˚C.

Animals

Twenty-one male C57BL/6 (6-week-old) mice were purchased from SLAC Laboratory Animal Technology Co., Ltd. (Shanghai, China). All mice were fed with food and water in the specific pathogen-free conditions through ordered humidity, temperature (22^{o}C) and a 12-h light/dark cycle. The animal experimental procedures were carried out according to international ethical guidelines and the National Institutes of Health Guide regarding the care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee of Fujian University of Traditional Chinese Medicine.

Animal experimental protocol and BP measurement

Twenty-one male C57BL/6 (10-week-old) mice were used in the study. The mice were randomly divided into three groups: the control, Ang II+Saline, and Ang II+QDGs groups (n=7 for each group). The control group was infused with 0.9% NaCl and orally treated with saline. The Ang II+Saline group was infused with Ang II (Sigma, 500ng/kg/min dissolved in 0.9% NaCl) and orally treated with saline. The Ang II+QDGs group was infused with Ang II (Sigma, 500ng/kg/min dissolved in 0.9% NaCl) and orally treated with 28.63mg QDG of per mouse every day. The infusions were executed with implanted mini-osmotic pumps (Alzet, model 2004) as described by Callis TE (Callis and Wang, 2008). All mice were infused and gavaged for 4 weeks. The SBP and DBP of all mice were measured on days 0, 1, 3, 5, 7, 14 and 28 by using the tail-cuff plethysmograph method and the CODA™ noninvasive blood pressure system (Kent Scientific, Torrington, CT, USA).

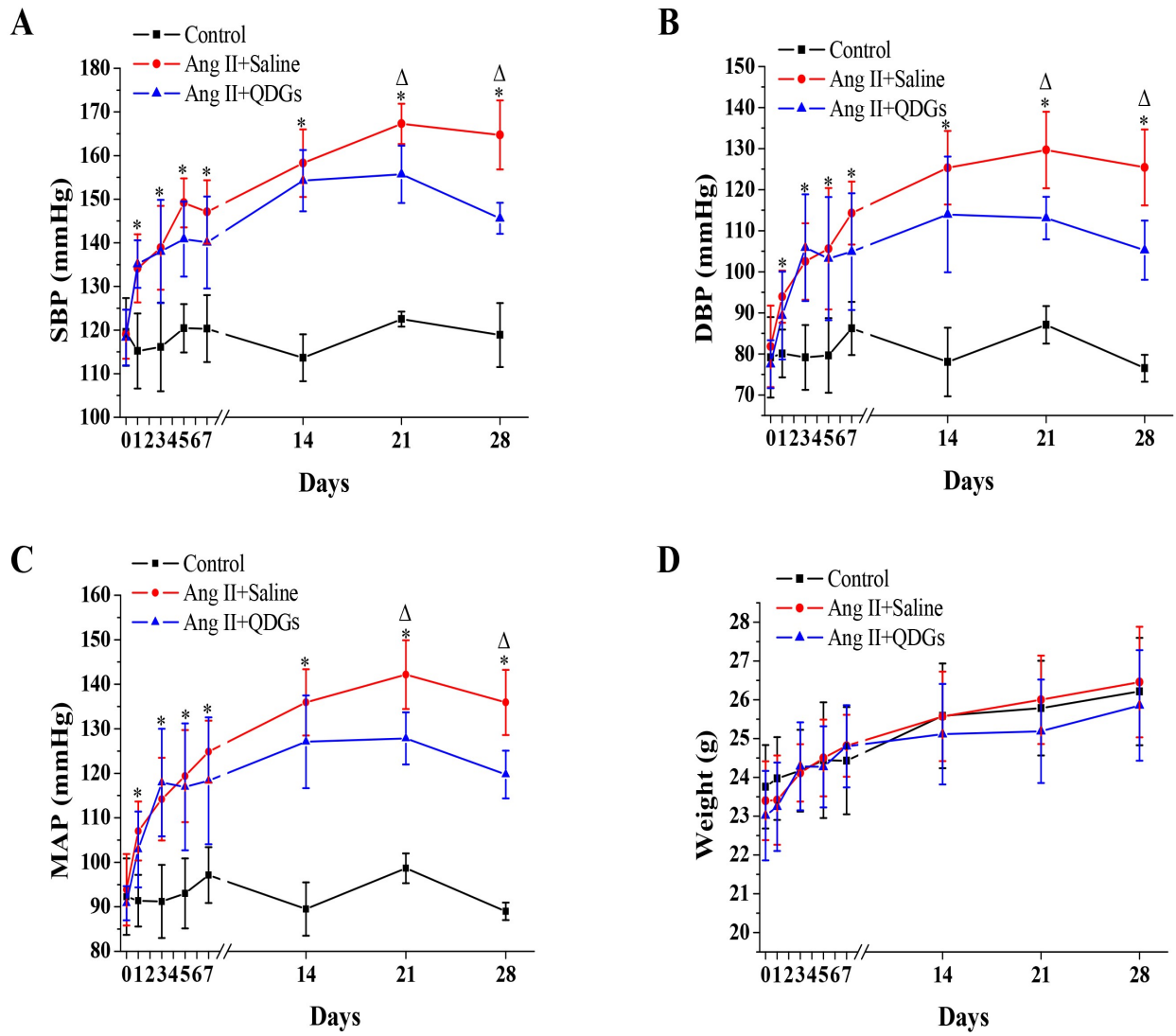

Fig. 1: Effect of QDGs treatment on blood pressure and body weight in Ang-II-mediated hypertensive mice. (A) SBP of Control, Ang-II+Saline, and Ang-II+QDGs groups. (B) DBP of Control, Ang-II+Saline and Ang-II+QDGs groups. (C) MAP of Control, Ang-II+Saline, and Ang-II+QDGs groups. (D) Body weight of Control, Ang-II+Saline and Ang-II+QDGs groups. * P<0.01, Ang-II+Saline group vs. Control group; Δ P<0.01, Ang-II+Saline group vs. Ang-II+QDGs group. QDGs, Qingda granules; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Control group, the saline infused mice treated with saline; Ang-II+Saline group, the Ang-II-infused mice treated with saline; Ang-II+QDGs group, the Ang-II-infused mice treated with QDGs.

Tissue preparation

The seven mice of each group were sacrificed by administering 2% pentobarbital sodium. The eyes of mice were removed and the blood was collected. Thus, the brains were removed quickly. The left brain of each mouse was perfused with 4% paraformaldehyde solution for 48 h to examine for hematoxylin-eosin (HE) staining and immunohistochemical (IHC) studies.

Serum isolation

Blood samples were collected in a tube without anticoagulants and allowed to rest at room temperature for 60 min. The clot was centrifuged at 1500 rpm for 15 min twice, and the resulting supernatant was separated to another tube that was designated serum. Avoiding freeze-thaw cycles, the serum was divided into 50µl per tube following centrifugation. Then, the serum was immediately maintained at -80°C.

Cerebral histopathology

The brains were routinely fixed for paraffin-embedded specimen and cut into a series of adjacent 5-µm thick slide. Then slides were stained and differentiated by hematoxylin and alcohol respectively and further stained with eosin for another 1 min. Pathological changes in brain tissue were observed under a light microscope after gradient dehydration and drying.

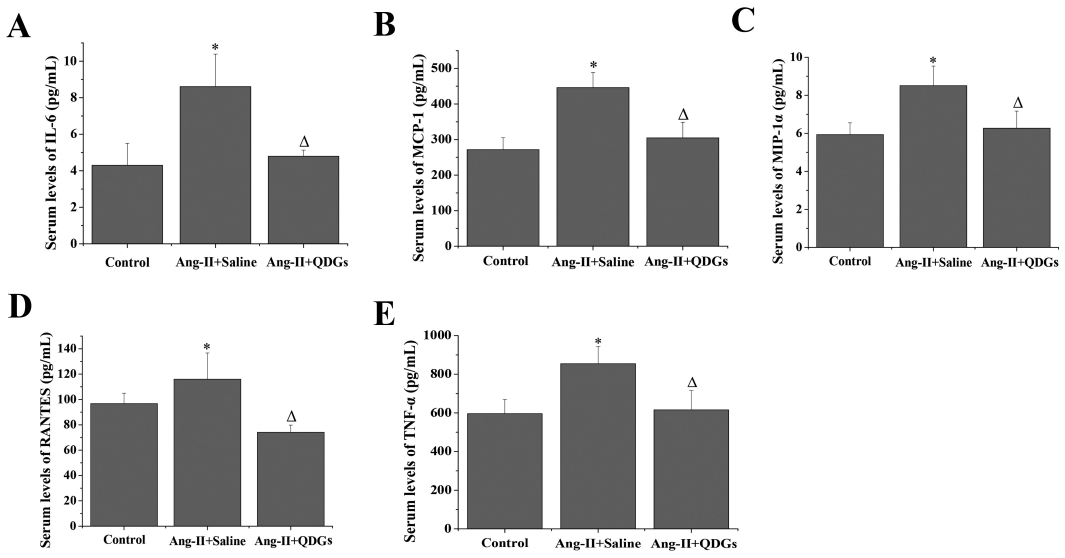

Fig. 2: Effect of QDGs treatment on the expression of cytokines (IL-6 (A), TNF-α (E)) and chemokines (MCP-1(B), MIP-1α (C), RANTES (D)) by bio-plex array system in Ang-II-mediated hypertensive mice. All values were represented as mean ± SD. *P<0.01, Ang-II+Saline group vs. Control group; ΔP<0.01, Ang-II+QDGs group vs. Ang-II+Saline group. QDGs, Qingda granules; Control group, the saline-infused mice treated with saline; Ang-II+Saline group, the Ang-II-infused mice treated with saline; Ang-II+QDGs group, the Ang-II-infused mice treated with QDGs.

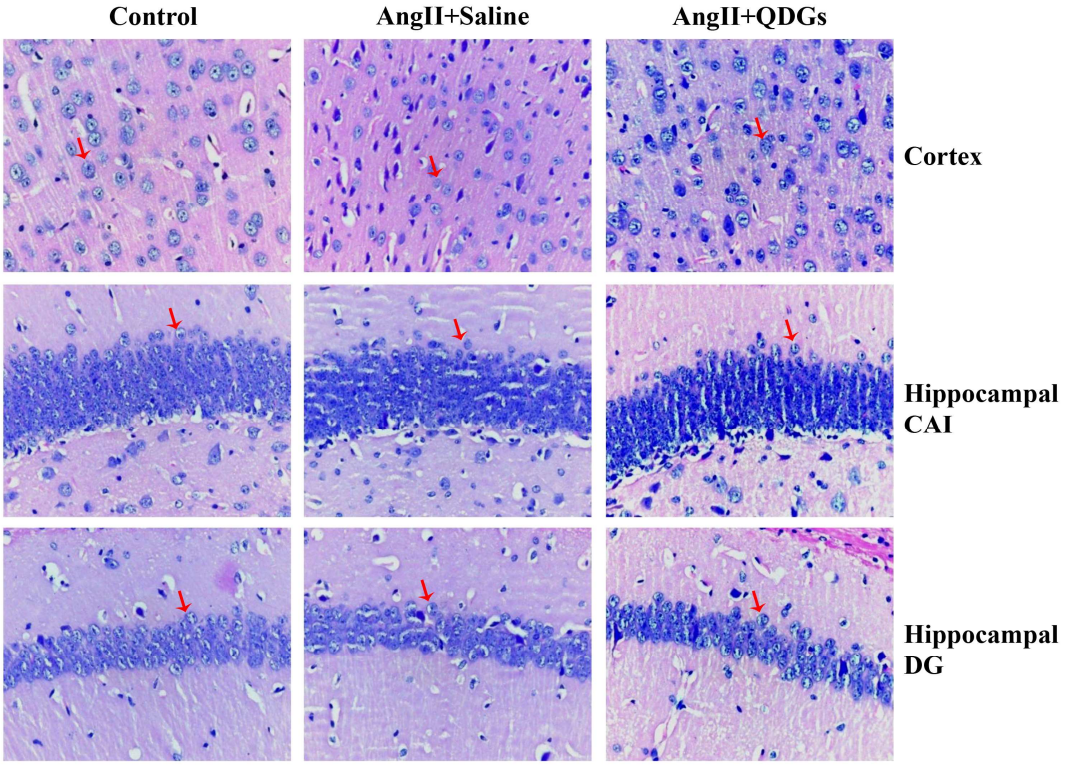

Fig. 3: Effect of QDGs treatment on the histopathologic change of brain by HE staining in Ang-II-mediated hypertensive mice. Histopathological changes in the cortex and hippocampal CAI, DG areas of the brain in each group were observed. Representative pictures are shown and taken at a magnification of 40×. QDGs, Qingda granules; HE, hematoxylin and eosin staining; Control group, the saline-infused mice treated with saline; Ang-II+Saline group, the Ang-II-infused mice treated with saline; Ang-II+QDGs group, the Ang-II-infused mice treated with QDGs.

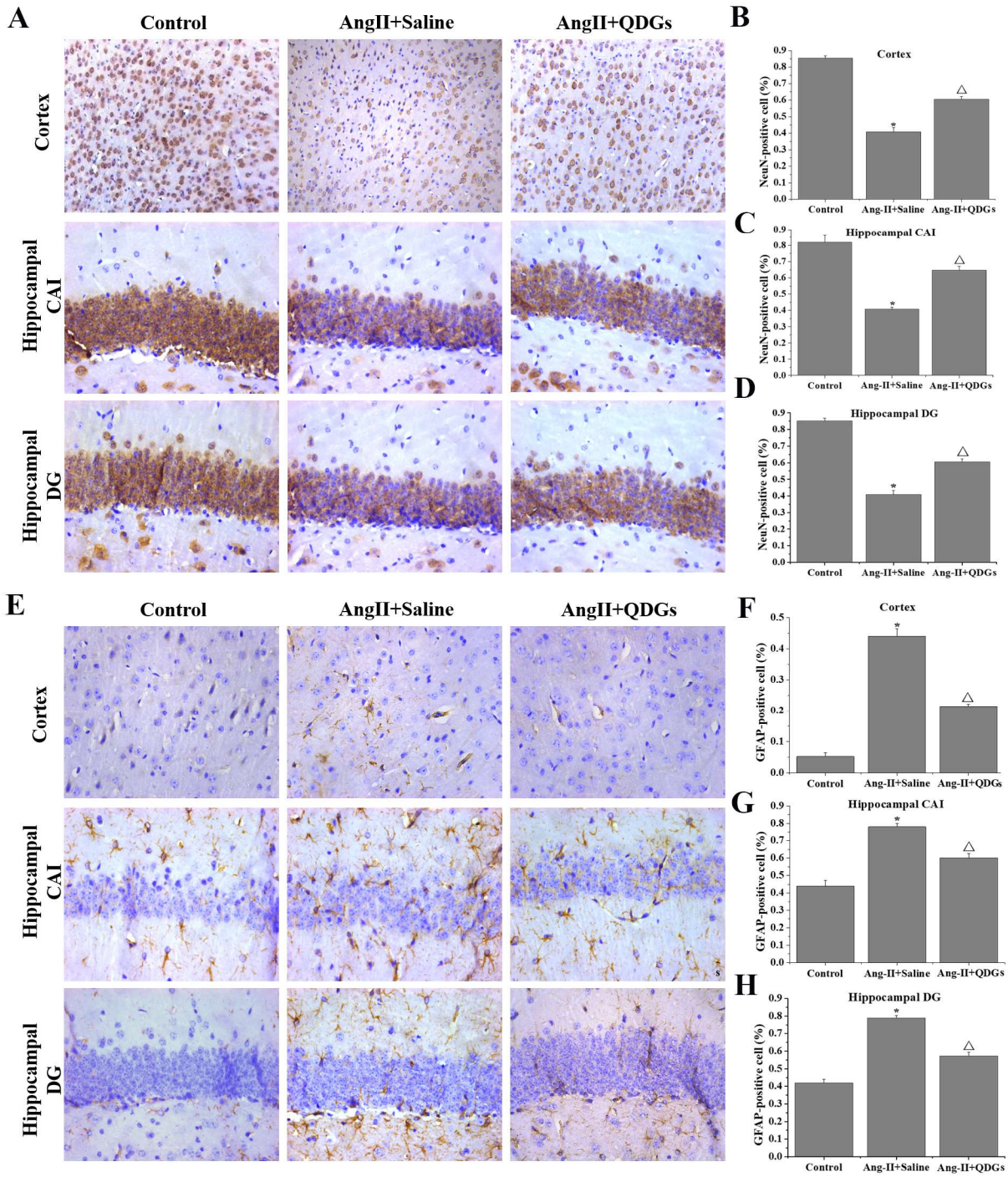

Fig. 4: Effect of QDGs treatment on the inhibition of loss of neurons and activation of astrocytes in the brain of Ang-II-mediated hypertensive mice. (A) Microphotographs of NeuN-positive neurons in the cortex and hippocampal CAI, DG areas of the brain. Representative pictures are shown and taken at a magnification of 40×. (B–D) Quantification of NeuN immunoreactivity by the percentage of positive cells in the cytoplasm. (E) Microphotographs of GFAP-positive astrocytes in the cortex and hippocampal CAI, DG areas of the brain. Representative pictures are shown and taken at a magnification of 40×. (F–H) Quantification of GFAP immunoreactivity by the percentage of positive cells. All values were represented as mean ± SD. *P<0.01, Ang-II + Saline group vs. Control group; ΔP<0.01, Ang-II+QDGs group vs. Ang-II+Saline group. QDGs, Qingda granules; IHC, immunohistochemistry; Control group, the saline-infused mice treated with saline; Ang-II+Saline group, the Ang-II-infused mice treated with saline; Ang-II+QDGs group, the Ang-II-infused mice treated with QDGs.

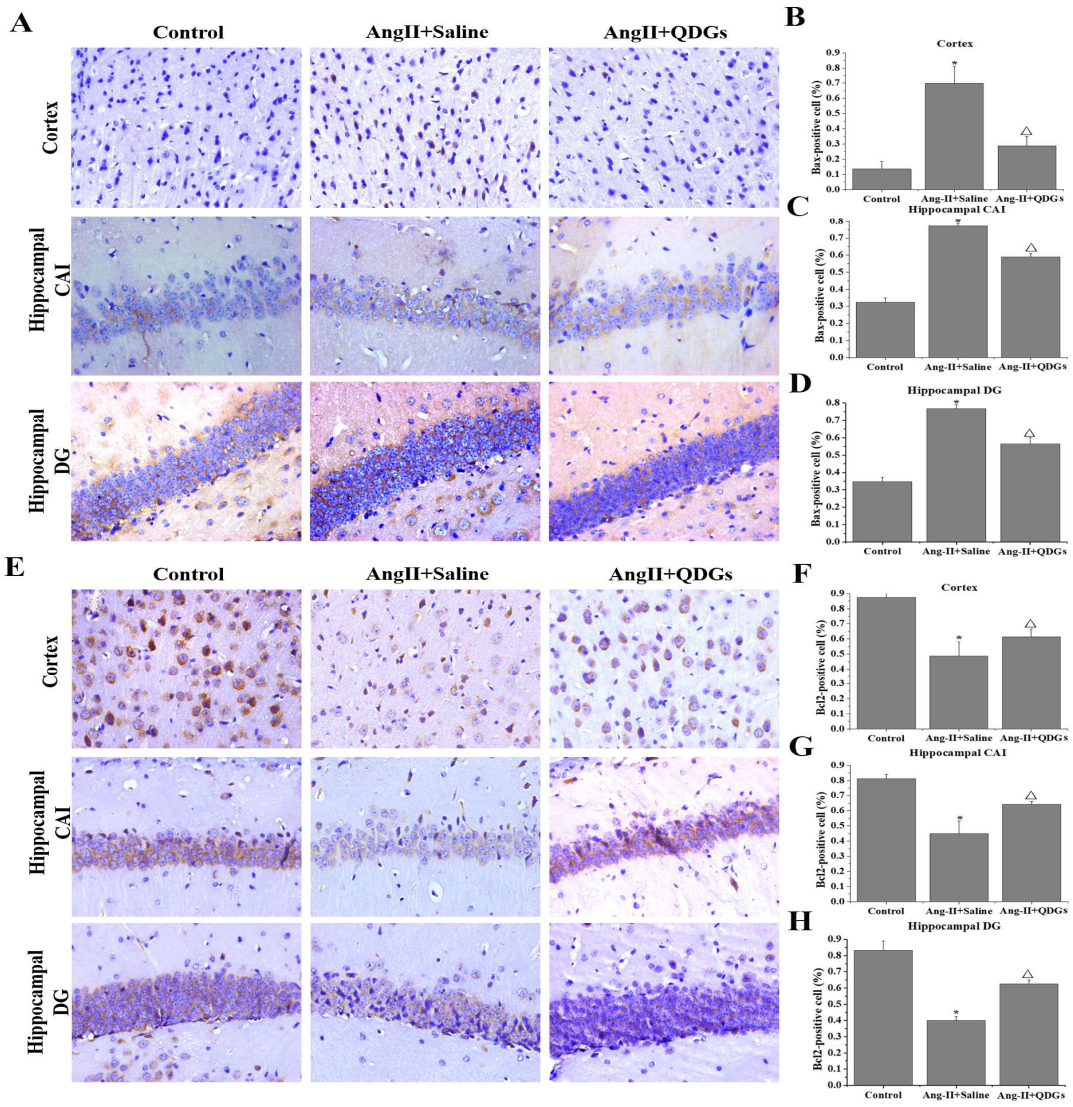

Fig. 5: Effect of QDG treatment on the regulation of Bax and Bcl-2 expression in the brain of Ang-II-mediated hypertensive mice. (A) IHC analysis was performed to determine the protein expression of Bax in the cortex and hippocampal CAI, DG areas of the brain. Representative pictures are shown and taken at a magnification of 40×. (B–D) Quantification of Bax expression by the percentage of positive cells in the cytoplasm. (E) IHC analysis was performed to determine the protein expression of Bcl-2 in the cortex and hippocampal CAI, DG areas of the brain. Representative pictures are shown and taken at a magnification of 40×. (F–H) Quantification of Bcl-2 expression by the percentage of positive cells in the cytoplasm. All values were represented as mean ± SD. *P<0.01, Ang-II+Saline group vs. Control group; ΔP< 0.01, Ang-II+QDG group vs. Ang-II+Saline group. QDG, Qingda granule; IHC, immunohistochemistry; Control group, the saline-infused mice treated with saline; Ang-II+Saline group, the Ang-II-infused mice treated with saline; Ang-II+QDG group, the Ang-II-infused mice treated with QDG.

IHC analysis

Slides (5-μm) were collected from above paraffin. The slides were blocked with 3% hydrogen peroxide, washed with PBS buffer, and then incubated with goat serum. The primary antibodies (NeuN (1:1000; Abcam, Cambridge, MA, UK), GFAP (1:500; Cell Signaling Technology, Beverly, MA, USA), Bax (1:100; Abcam, Cambridge, MA, UK), and Bcl-2 (1:200; Cell Signaling Technology, Beverly, MA, USA) and HRP-labeled secondary antibody (Maixing, Fuzhou, China), were then incubated for those slices respectively. Finally, the slices were stained with DAB and counterstained with hematoxylin. Six microscopic fields were choose randomly for each slide, then protein expression was quantified by the percentage of positive cells by using image analysis system (Image-Pro Plus, Media Cybernetics). For avoiding non-specific staining, PBS was served as the negative control instead of primary antibody.

Multiplex cytokine assays

The cytokine array assay was performed in the serum of each mice via a multiplex cytokine assay system (Bio-rad). Twenty-three cytokines/chemokines, including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, and TNF-α were quantified in the serum of all experimental mice. The assays were performed according to the manufacturers' instructions. The fluorescence intensity of each bead was determined via a Bio-plex array reader.

STATISTICAL ANALYSIS

All data were expressed as the mean ± standard deviation (SD). Statistical significance was calculated by using one-way analysis of variance followed by SPSS software (version 21; SPSS, Inc., Chicago, IL, USA). P values <0.05 were considered as statistically significant.

RESULTS

QDGs attenuated the elevation of BP in Ang II-mediated hypertensive mice

To determine the effect of QDGs against hypertension, mouse model of Ang II-mediated hypertension was constructed. Baseline SBP, diastolic BP (DBP), and mean arterial pressure (MAP) were measured for C57/BL6 mice, and no significant differences in these measurements between the model mice and control mice were found. After the first day of construction, the SBP, DBP, and MAP values of the Ang II model mice were 134.1±7.8mmHg; 94.0±6.3mmHg, and 107±6.6mmHg, respectively, which were obviously higher than those in the control group (those not infused with Ang II; SBP, 115.2±10.7mmHg; DBP, 80.1±5.8 mmHg; MAP, 90.4 ± 5.8 mmHg). This finding indicated that the mouse model of Ang II-mediated hypertension had been successfully established. The mice were then randomized into two groups: The Ang II+Saline- and Ang II+QDGs-treated groups. After the 21^{rst} day of QDGs treatment, the Ang II+QDGs-treated group showed marked therapeutic efficacy in controlling SBP (155.7±6.6mmHg), DBP (113.1±5.2mmHg), and MAP (127.8±5.8mmHg) when compared with the Ang II+Saline group, which had higher SBP (167.3±4.6mmHg), DBP (129.7±9.3mmHg), and MAP (142.2±7.7mmHg) (figs. 1A-C).

In addition, if there are some toxic compounds in the QDGs, the mice will lose weight after daily oral administration of QDGs. For this reason, we further examined the effect of QDGs on the weight of mice. As shown in fig. 1D, an age-related increase in body weight was observed in three groups, and there was no significant difference among the Ang II+Saline, Ang+QDGs, and control groups. Collectively, the results showed that QDGs attenuated elevated BP and had no obviously adverse effects in Ang II-mediated hypertensive mice.

QDGs ameliorated inflammation in Ang II-mediated hypertensive mice

To determine if QDGs had anti-hypertensive effects caused by decreasing inflammation, we performed a bio-plex array to detect the serum levels of 23 potentially associated cytokines/chemokines, including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, and TNF-α, in Ang II-mediated hypertensive mice. As shown in fig. 2, the levels of cytokines (IL-6, TNF-α) and chemokines (MCP-1, MIP-1α, RANTES) were significant increases in the serum of the Ang II+Saline group relative to those in the serum of the control group. Of note, after QDGs treatment, the serum levels of IL-6, TNF-α, MCP-1, MIP-1α, and RANTES were obviously downregulated in the Ang II-mediated hypertensive mice. These results suggested that QDGs treatment attenuated the elevation of BP potentially by inhibiting inflammation.

QDGs improved cerebral histopathology in Ang II-mediated hypertensive mice

Brain injury, accompanied by a decrease in neurons and abnormally activated glial cells, is a common symptom during the process of hypertension (Girouard and Iadecola, 2006). To demonstrate the protective effect of QDGs on brain injury of Ang II-mediated hypertensive mice, we analyzed the change in cerebral histopathology by HE staining. As shown in fig. 3, the cortex and hippocampal cornu ammonis CAI and dentate gyrus (DG) areas exhibited abundant neurons that were eumorphic and distributed uniformly and a small amount of normal glial cells in the control group. In contrast, the cell morphology of most neurons in the Ang II+Saline group showed disorder, shrinkage, nuclear pyknosis, and vacuolization as well as a decrease in neurons and an increase in glial cells. Of note, after treatment with QDGs, the extent of damage was significantly diminished and the neurons appeared relatively normal. The results preliminarily indicated that QDGs improved cerebral histological changes.

QDGs alleviated the loss of neurons and activation of astrocytes in Ang II-mediated hypertensive mice

To further determine if QDGs could ameliorate the status of neurons and astrocytes in brain injury of Ang-II-mediated hypertensive mice, we performed IHC analysis to examine the expression of NeuN (a sensitive and specific neuronal marker) and GFAP (a standard marker of astrocytes) in the cortex and hippocampal CAI and DG areas. As shown in fig. 4, the neurons mainly survived in the control group, whereas the reduction in NeuN in immunostaining was observed in the cortex and hippocampal CAI and DG areas of the Ang II+Saline

group, a finding that has been considered to be evidence of neuronal death in some researches. In the Ang II+QDGs-treated group, the abundance of NeuN-positive neurons in the cortex and hippocampal CAI and DG areas was obviouslsy higher than that in the Ang II+Saline group. The astrocytes displayed reactive changes in the cortex and hippocampal CAI and DG areas of the Ang II+Saline group, which were considered to have upregulated the expression of GFAP. QDGs treatment could reduced the activations of astrocytes in these areas. Immunostaining of NeuN and GFAP were consistent with the observations from HE staining. These results demonstrated that QDGs treatment significantly inhibited neuronal death and activations of astrocytes in the brain.

QDGs inhibited apoptosis in Ang-II-mediated hypertensive mice

Apoptosis is one of the major pathways that contributes to neuronal death via regulation by anti-apoptotic members of the Bcl-2 family (Mattson *et al.*, 2001, Lawen, 2003). In this study, IHC analysis was performed to evaluate the expressions of pro-apoptotic Bax and anti-apoptotic Bcl-2 in these groups. Compared with the control group, the expression of Bcl-2 and Bax were significatly downregulated and upregalated respectively in the cortex and hippocampal CAI and DG areas at the Ang-II+Saline group. Conversely, the Ang II+QDGs-treated group show the opposite result of the expression of Bcl-2 and Bax in these areas (fig. 5). Taken together, these results suggested that the QDGs could inhibit apoptosis in brain injury of Ang II-mediated hypertensive mice.

DISCUSSION

Hypertension is a complicated, multifactorial, polygenic disease that develops target-organ comorbidity. Numerous drugs, including diuretics, sympathoplegic agents, renin inhibitors, ACE inhibitors, angiotensin receptors blockers and so on, have been developed to control elevated clinical BP(Goodnow, 2007). Unfortunately, these drugs exhibit therapeutic effects for only 34% of hypertensive patients (Wang and Xiong, 2012). Taking this into account, the number of patients seeking herbal remedies for the treatment and management of hypertension, has greatly increased dramatically over the past decade. (Frishman *et al.*, 2009).

Obviously, the reasonable use of herbal remedies is certainly expected, considering that they have thousands of bioactive compounds with the potential therapeutic effects (Disi *et al.*, 2016, Anwar *et al.*, 2016). TCM has been practiced in China for long time as essential remedies for hypertension with few undesired toxicities. QXJYD, prescribed by academician Ke-ji Chen, has been used in BP control and achieved good clinical outcomes in hypertensive patients for >60 years. In animal experimental studies, QXJYD has shown its anti-hypertensive effect as well as reversing vascular remodeling and mitigating renal interstitial fibrosis in spontaneously hypertensive rats (Xiao *et al.*, 2016, Liu *et al.*, 2017). Although QXJYD exhibits excellent anti-hypertensive properties, the 10-herb formula is complicated for development as a new pharmaceutical. QDGs, which are a simplified form of QXJYD, exhibited suppressive effects on the increased SBP, DBP, and MAP in the Ang II-mediated hypertensive mice. Moreover, QDGs showed less side effects since the formulation had no influence on the changes of experimental animals' body weight over the entire process of the study. Above all, QDGs might serve as a novel anti-hypertensive pharmaceutical. However, the functional mechanism of QDGs on anti-hypertensive properties remains unclear.

Some studies has been implied that Ang II elevates vascular permeability to trigger the inflammation and recruit inflammatory cells into tissue, which could directly activate infiltrating immunocompetent cells (Nataraj et al., 1999, Piqueras et al., 2000). Recently, evidence has been mounting indicating that process of inflammation involves in the development of hypertension (Cohenkaminsky et al., 2014). Inhibition of inflammation can effectively treat hypertension or slow down the progression of hypertension and reduce the damage to target organs of hypertension (Savoia and Schiffrin, 2006). Ang II increases vascular permeability that results in cell infiltration and exudation of protein-rich fluid and initiation of the inflammatory process via the release of prostaglandins and VEGF or rearrangement of cytoskeletal proteins (Nataraj *et al.*, 1999). Ang II has been shown to strongly influence cell adhesion, transmigration, and chemotaxis of inflammatory cells into the tissue by regulating the expression of adhesion molecules and chemokines (Piqueras *et al.*, 2000). The inflammatory process is a key event in the development of hypertension (Cohenkaminsky *et al.*, 2014). Inhibition of inflammation can effectively treat hypertension or slow down the progression of hypertension and reduce the damage of target organs of hypertension (Savoia and Schiffrin, 2006). In our experiment, QDGs decreased the expressions of several pro-inflammatory mediators (IL-6, TNF-α, MCP-1, MIP-1α, and RANTES). These cytokines/chemokines are all known to show higher expression in Ang II-mediated hypertensive mice. Bautista LE reported that IL-6 and TNF-α could be highly independent risk factors for high BP in healthy people and found that higher expression of these cytokines was positively associated with damage to target organs of hypertension (Bautista *et al.*, 2005, Savale *et al.*, 2009). In addition, C-C chemokines participate in mild acute or chronic inflammation through monocytes and lymphocyte (Wenzel and Abboud, 1995). Ang II-enhanced C-C chemokines (such as MCP-1, MIP-1α, and RANTES) have been reported to have major roles in hypertension development. Numerous experimental models of hypertension have indicated that RAS blockade decreased MCP-1 expression in circulating monocytes to ameliorate

monocyte/macrophage infiltration into aortic tissues (Dol *et al.*, 2001). Taken together, these results demonstrated that QDGs treatment significantly attenuating inflammation might be a potential functional mechanism against Ang II-mediated hypertension.

Hypertension is a major risk factor for stroke due to the harmful effects on the brain and its vessels (Pires *et al.*, 2013). A continuous blood supply was required to maintain brain function. The alterations of cerebral artery structure and function caused by hypertension results in decreased blood flow (Pires *et al.*, 2013, Farkas *et al.*, 2000). Additionally, changes in cerebral artery structure also block nutrients and oxygen from delivery to neurons. Finally, the pathological changes in neurons and neurometabolic disorder have been found (Farkas *et al.*, 2000). Additionally, inflammation, oxidative stress, and vasoactive circulating molecules disorder blood–brain barrier in the development of hypertension (Abbott *et al.*, 2010). Neuronal loss, cognitive deficit, and impaired recovery from ischemia occurred mainly due to neurons exposure to cytotoxic molecules (Dénes *et al.*, 2011, Torre, 2012). During the process of injury in the brain, glial cells are rapidly activated and their morphology and function are altered (Park *et al.*, 2008). In our experiment, we observed that QDGs treatment significantly improved the cerebral histological changes, such as by increasing the number of neurons and reversing the state of neurons that are typically characterized by disorder, shrinkage, nuclear pyknosis, and vacuolization in the cortex and hippocampal CAI and DG areas of the Ang II+Saline group. Finally, the increase in abnormal activated glial cells was also suppressed after QDGs treatment.

Neurons are the crucial components of the brain and their morphology and function are indispensable for the nervous system. NeuN is considered to be an effective and specific marker for neurons (Davoli *et al.*, 2002). It has been revealed that loss of NeuN in mouse brains was associated with an increase in neuronal apoptosis (Davoli *et al.*, 2002, Sugawara *et al.*, 2002). GFAP plays a pivotal role in regulating the motility and shape of astrocyte by providing structural stability for the process of astrocyte. In response to brain injury, astrocytes turn reactive and respond in a typical manner, named astrogliosis. Reactive astrogliosis is a major feature of astrocytes to brain injury. Astrogliosis is considered to increase synthesis of GFAP via up-regulating the protein expression (Eng *et al.*, 2000). In our study, QDGs treatment significantly alleviated the loss of neurons and the activation of astrocytes in the cortex and hippocampal CAI and DG areas of the brain, which is consistent with the histopathological results.

Apoptosis is one of the major pathways that contributes to neuronal death, and mitochondria are core cellular organelles of energy generation that have a pivotal role in apoptotic signaling in injury (Mattson *et al.*, 2001, Zuo *et al.*, 2014). The pathway is strictly regulated by the anti-apoptotic members of the Bcl-2 family, and their aberrant expression impairs the normal apoptotic program (Lawen, 2003). During the process, a pro-apoptotic protein, such as Bax, translocates from cytosol to mitochondria, which results in mitochondrial membrane permeabilization that finally leads to cell death (Kuwana *et al.*, 2002). In normal neurons, Bax is isolated by Bcl-2 in an attempt to prevent pore formation on the mitochondrial membrane and suppress apoptosis. Therefore, a higher Bax-to-Bcl-2 ratio implies the development of apoptosis (Torre, 2012). In the study, a significant down-regulation in Bcl-2 expression and up-regulation in Bax expression was detected in the Ang II-mediated hypertensive mice brains. However, QDGs reversed the higher Bax-to-Bcl-2 ratio via increasing the expression of Bcl-2 and decreasing the expression of Bax. The present study suggested that inhibition of neuronal death via mitochondrial apoptotic signaling may be one of the potential mechanisms of QDGs against Ang II-induced brain injury.

CONCLUSION

In summary, we demonstrated for the first time that QDGs attenuated elevation of BP possibly via inhibition of inflammation. QDGs promote neuronal survival and alleviate activation of astrocytes to exert neuroprotective effects against Ang II-induced brain injury. Above all, our findings indicate that QDGs are an effective and promising therapeutic agent for the treatment of Ang II-mediated hypertension. However, QDGs have a pivotal role in anti-hypertension through various signaling pathways, but the other underlying molecular mechanisms need to be further elucidated.

ACKNOWLEDGEMENTS

This work was sponsored by National Natural Science Foundation of China (81774135), Science and technology platform construction project of Fujian science and Technology Department (Grant No. 2015Y2001), Fujian Province Natural Science Foundation (2019J01357).

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