Gastrodin Attenuates Bilateral Common Carotid Artery Occlusion-Induced Cognitive Deficits via Regulating Aβ-Related Proteins and Reducing Autophagy and Apoptosis in Rats

Bo Liu1,2, Jian-Mei Gao2, Fei Li2, Qi-Hai Gong2 and Jing-Shan Shi2*

1 School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, China, 2 Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, China

Gastrodin (GAS), an active constituent extracted from Gastrodia elata Blume, is used to treat ischemic stroke, epilepsy, dizziness, and dementia for centuries in China. This study examined its effects on vascular dementia (VD) and the underlying molecular mechanisms. VD was established by ligation of bilateral common carotid artery occlusion (BCCAO). A total of 7 days after BCCAO surgery, GAS (15, 30, and 60 mg/kg) was orally administered for 28 consecutive days to evaluate therapeutic effects. Cognitive function was tested by the Morris water maze. The neuronal morphological changes were examined via Hematoxylin–Eosin staining. Flow cytometry was used for evaluating apoptosis in the hippocampi. The target protein expression was examined by Western blot. The results showed that BCCAO induced cognitive impairment, hippocampus CA1 and CA3 pyramidal neuron damage, beta-amyloid (Aβ) deposition, excessive autophagy, and apoptosis. GAS treatment significantly improved BCCAO-induced cognitive deficits and hippocampus neuron damage. Molecular analysis revealed that GAS exerted the protective effect via reducing the levels of Aβ1–40/42, APP, and β-site APP-cleaving enzyme 1 expression, and increasing Aβ-related protein, a disintegrin and metalloprotease 10, and insulin degrading enzyme expression. Meanwhile, GAS inhibited excessive autophagy via decreasing Beclin-1, LC3-II, and p62 levels. Furthermore, GAS inhibited apoptosis through the downregulation of Bax and upregulation of Bcl-2. Moreover, P38 MAPK signaling pathway was involved in the process. Our findings demonstrate that GAS was effective in the treatment of BCCAO-induced VD via targeting Aβ-related protein formation and inhibiting autophagy and apoptosis of hippocampus neurons.

Keywords: gastrodin, cognitive deficits, hippocampus, Aβ-related proteins, autophagy, apoptosis
INTRODUCTION

Vascular dementia (VD), a kind of acquired intelligence damaging syndrome, is characterized by neurodegeneration, cognitive impairment, and memory difficulty. With a considerable growth of elderly population, VD is becoming the second most common type of dementia after Alzheimer’s disease (AD). Unfortunately, up to now, effective therapeutic approaches are unavailable (Smith, 2017). With an urgent demand for novel neuroprotective strategies to treatment of VD, numerous studies have been taken to search effective therapies. Among these studies, bilateral common carotid artery occlusion (BCCAO) in rats is widely accepted as an experimental model, which can imitate the pathological change occurred in VD successfully (Jiwa et al., 2010).

Currently, the underlying mechanisms of VD have been linked to hippocampus neuron damage, inflammatory response, and oxidative stress (Jiwa et al., 2010; Zhang et al., 2018). Furthermore, BCCAO-induced cognitive impairment and the occurrence of VD-like pathogenesis characterized by the deposition of beta-amyloid (Aβ) in the hippocampus are found (Cai et al., 2017), and emerging strategies for interfering with the metabolism of Aβ might be promising therapeutic approaches to attenuate or reverse negative neurological consequences of BCCAO (Won et al., 2013; Zhu et al., 2017). Autophagy is thought to contribute to the clearance of Aβ. However, either too much or too little autophagy is harmful to neuron. Growing evidence in support of abnormal autophagy is correlated with Aβ deposition after BCCAO. Aβ mediated autophagy flux and accumulation of autophagosomes, and the inhibition of autophagy decreased Aβ-induced cytotoxicity after BCCAO injury (Nagatani et al., 2012; Zou et al., 2017). Simultaneously, other mechanisms such as apoptosis of hippocampal neurons may be also triggered by the accumulation of Aβ (Zhang Y. et al., 2016; Xiong et al., 2017).

Due to the complexity of VD pathological processes, increasing interests have switched to the natural products derived from herbs, which exert multi-target effects, less adverse drug reactions. Gastrodia elata Blume, commonly called Tianma (天麻) in Chinese, described to enter the liver meridian used for calming liver to stop endogenous Wind. Gastrodin (GAS; C_13H_16O_7; molecular weight: 286.28; purity ≥ 98%); was purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China). GAS was dissolved in normal saline (NS). All reagents were of analytical-reagent grade and were generally commercially available.

Thus, the present study was designed to investigate whether GAS exert beneficial to BCCAO-induced cognitive impairment, and explore its underlying mechanisms, focusing on Aβ-related proteins, and autophagy and apoptosis.

MATERIALS AND METHODS

Drugs
The GAS (C_13H_16O_7; molecular weight: 286.28; purity ≥ 98%) was purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China). GAS was dissolved in normal saline (NS). All reagents were of analytical-reagent grade and were generally commercially available.

Animals
Adult male Sprague-Dawley rats weighing 260 ± 20 g were purchased from the Experimental Animal Center of the Third Military Medical University (SPF grade, Certificate No. SCXK2007-0005). The study protocol was approved by the Experimental Animal Ethics Committee at the Zunyi Medical University. All animals were allowed adaptive feeding for a week prior to experimentation. Rats were housed under a 12 h light/dark cycle at 22–24°C with free access to food and water. Efforts were made to minimize the number of animals tested and their suffering. Animals were randomly divided into the following six groups: Sham, Sham + GAS (60 mg/kg), BCCAO, and BCCAO + GAS (15, 30, and 60 mg/kg) groups, respectively. All animals were orally gavaged with GAS or saline daily at the seventh day after surgery for 28 days.

Surgery
The BCCAO model in rats was carried out as previously reported (Li et al., 2015; Zhang Y. et al., 2016). Briefly, after deep anesthesia with 2% sodium pentobarbital (3 ml/kg, i.p.), the bilateral common carotid arteries of the animals were exposed through a midline incision in the neck and carefully separated from the peripheral tissues, then ligated with surgical silk. The operations were performed on a heating pad to maintain body temperature of rats at 37.5 ± 0.5°C, and the rats were kept on the pad until recovery from anesthesia.

Morris Water Maze
Spatial learning and memory of all rats were evaluated using the Morris water maze (MWM) test as described earlier (Hu et al., 2017). Place navigation test was performed from day 24 after operation for 4 consecutive days (Figure 1A). In brief, the circular heated water pool was in the diameter 120 cm and height 50 cm, water temperature of 24–26°C was maintained inside, and 1 kg of powdered milk was added to make the water opaque. All external visual clues surrounded by the pool were kept constant for the spatial orientation of the rats. A platform was submerged 2 cm below the water surface in the middle of the northwest quadrant.

Each animal was softly put into water at one of the four starting positions facing the wall of the pool. The swimming time of each rat from the start location to reach the submerged platform (escape latency) was recorded. If the animal missed...
the hidden platform within the specified time, it was guided to stay on the platform for 15 s and the escape latency was recorded as 120 s. On the fifth day, the platform was removed and the rat was allowed to swim in the pool freely for 120 s, the time and frequency spent in the target quadrant were measured. Performance was taken notes by a computer-based video tracking system and analyzed by the MT-200 image analyzing software (Taimeng Co., Chengdu, China).

**Hematoxylin–Eosin (HE) Staining**

Rats were sacrificed after the MWM, and brains were perfused by a tip transfusion pump through which inserted from apex area of heart into the aorta perfusion, perfused with 0.9% cold phosphate buffered saline and 4% paraformaldehyde. After that, the brain tissues were removed and 4% paraformaldehyde was used to incubate for 24 h at 4°C. Brain samples behind optic chiasm including the whole hippocampus were removed to embed in paraffin. Embedded brain tissue sections were continuous sliced into 4–5 μm sections for histological analysis according to the instructions. Light microscopy was used to observe the histomorphology of neurons, and CA1 and CA3 subfields of hippocampi counted at 400× magnification were selected to check for morphological alterations.

**Flow Cytometry With AnnexinV-FITC/PI Double Staining**

Flow cytometry analysis of tissue was performed as described previously (Schutte et al., 1998; Erising and Tekellioglu, 2017; Kim et al., 2017). Annexing-FITC/PI double staining was performed and the apoptosis rate of hippocampi was tested with flow cytometry. Hippocampal tissue was added with 500 μL physiological saline, grinded with a glass rod, and the cell suspension was filtered through a 200-mesh sieve. Washed with pre-cooled PBS for three times, cells were centrifuged for removal of the supernatant. Cells were re-suspended with 500 μL binding buffer, reacted with 5 μL annexin V-FITC and 5 μL PI at room temperature for 5–10 min in the dark, and then quantified using flow cytometry ( Beckman Coulter, United States) at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

**Western Blot Analysis**

After rats were sacrificed, the isolated hippocampal tissues were used for Western blot analysis. The tissues were homogenized in ice-cold lysis buffer supplemented with protease inhibitors and phosphatase inhibitors. The tissues were centrifuged at 4°C for 15 min, and supernatant was collected. Protein concentration was measured using the BCA assay kit. Then, the lysates were separated with 5–12% SDS–PAGE and transferred to PVDF membranes. Followed by electrophoresis, PVDF membranes were blocked by 5% non-fat milk at room temperature. After that, membranes were incubated overnight at 4°C with primary antibodies against the following proteins: Aβ 1–40 (Aβ1–40, 1:1000, Abcam, United States), Aβ 1–42 (Aβ1–42, 1:1000, Abcam, United States), amyloid precursor protein (APP, 1:1000, Sangon Biotech, China), β-site APP-cleaving enzyme 1 (BACE1, 1:1000, Sangon Biotech, China), disintegrin and metalloprotease 10 (ADAM10, 1:1000, Abcam, United States), insulin degrading enzyme (IDE, 1:1000, Abcam, United States), autophagy-related protein Beclin-1 (Beclin-1, 1:1000, Abcam, United States), autophagy marker Light Chain 3 (LC3, 1:1000, Novus Biologicals, United States), nucleoporin p62 (p62, 1:1000, Abcam, United States), phosphorylation of P38 mitogen-activated protein kinase (p-P38MAPK, 1:2000, Abcam, United States), P38 mitogen-activated protein kinase (P38MAPK, 1:2000, Abcam, United States), B cell lymphoma/leukemia-2 (Bcl-2, 1:2000, Abcam, United States), Bcl-2-associated X protein (Bax, 1:2000, Abcam, United States). Then, the membranes were incubated with the relevant secondary antibodies for 2 h. Blots were visualized using chemiluminescence reagent BeyoECL Plus (Beyotime). Quantity One 1-D analysis software v4.52 (BioRad) was used for scanning the image and quantifying band intensity.

**RESULTS**

**GAS Attenuates Learning and Memory Impairments in the BCCAO Rats**

The MWM was used to evaluate the spatial learning and memory function of GAS treatment. A training trial lasts for 4 days, and the escape latency time was recorded. As shown in Figure 1B, the animals in BCCAO group presented significantly prolonged escape latency than that of sham group (P < 0.01), indicating the impairment of the learning and memory performances representing in VD models. However, treatment with GAS significantly mitigated the poor spatial learning in the MWM test (P < 0.05). We also found Sham and Sham + GAS (60 mg/kg) did not show any change on learning and memory impairments. Subsequently on the fifth day, we performed a probe trial to evaluate the spatial memory retention among different groups. Results showed that rats in BCCAO group presented worse memory ability with spent less time in the platform quadrant than that of Sham and Sham + GAS (60 mg/kg) (P < 0.05). However, GAS (15, 30, and 60 mg/kg, respectively) treatments significantly arrested the spatial memory impairment (Figure 1C, P < 0.05).

**GAS Attenuates Neuronal Damage in the Hippocampus Induced by BCCAO**

The rat hippocampal CA1 and CA3 region was observed by HE staining (Figure 2). The results showed that most neurons were lost, shrinkage, dark-stained, and with severe cellular edema both in the CA1 and in the CA3 areas of the hippocampus in BCCAO group compared with Sham and Sham + GAS (60 mg/kg) groups. However, GAS-treatment groups attenuated BCCAO-induced neuronal damage, especially in the GAS (60 mg/kg) group.
FIGURE 1 | Effects of GAS on Morris water maze performance deficits induced by BCCAO in rats. (A) Schematic representation of the experimental design. (B) The latencies were measured to assess the rat learning and memory ability in 4 days training trials. (C) The percentage of time in the target quadrant. Data are presented as means ± SEM (n = 10). *P < 0.05; **P < 0.01 vs. sham; #P < 0.05; ##P < 0.01 vs. BCCAO.

FIGURE 2 | Effects of GAS on BCCAO-induced morphological alterations in the hippocampi CA1 and CA3 subfields after BCCAO (Magnification 400×, Scale bar = 50 μm). Representative sections were stained using HE. Normal cellular morphology was present in the Sham and Sham + GAS (60 mg/kg) groups. Compared with the BCCAO group, a gradual improvement in condensed nuclei (arrows) was detected in the hippocampal CA1 and CA3 region in each GAS treatment group.

GAS Suppresses Apoptosis in the BCCAO Rats

Analysis of BCCAO induced apoptosis in the hippocampus using Annexing-FITC/PI double staining assay, and the rate of apoptosis cells in the hippocampus of BCCAO was to determine the addition percentages of C2 (late apoptotic and necrotic cells) and C4 (early apoptotic cells). As is shown in Figure 3, BCCAO group was increased compared with Sham and Sham + GAS (60 mg/kg) groups (P < 0.01). GAS was able to inhibit the BCCAO-induced cell apoptosis in a dose-dependent manner (P < 0.01).

GAS Reduces Aβ1−40 and Aβ1−42 Level in the Hippocampus Induced by BCCAO

Western blot results (Figure 4) showed that a significant increase in the level of Aβ1−40/42 protein in the group of BCCAO rats (P < 0.05), as compared to those treated with or without GAS. However, when groups were administered with different concentrations of GAS simultaneously, the BCCAO-induced Aβ1−40/42 protein increase could be considerably prevented (P < 0.05).

GAS Regulates the Expression of Aβ-Related Protein in the Hippocampus of the BCCAO Rats

Further to explore the mechanisms of GAS on reduction of BCCAO-induced deposition of Aβ, the level of Aβ-related protein were detected using Western blot (Figure 5). The expressions of APP and BACE1 were significantly increased in BCCAO group compared with Sham and Sham + GAS (60 mg/kg) groups (P < 0.01). However, BCCAO-induced high expression of APP and BACE1 has been prevented by various doses of GAS. Meanwhile, the low expression of ADAM10 and IDE in BCCAO group was also significantly raised by GAS in different doses (P < 0.01).
GAS Regulates the Expression of Autophagy-Related Protein in the Hippocampus of the BCCAO Rats

To further confirm the effect of GAS on abnormal autophagy, we examined autophagy-related proteins in each group by Western blot (Figure 6). Beclin-1 and LC3-II were increased, simultaneously, p62 was decreased in the BCCAO group compared with that in Sham and Sham + GAS (60 mg/kg) group, but these effects were reversed by treatment with various doses of GAS ($P < 0.05$).

GAS Regulates the Expression of Apoptosis-Related Protein in the Hippocampus of the BCCAO Rats

As shown in Figure 7, BCCAO resulted in a significantly increase of p-P38 MAPK and Bax and a drastic decrease of Bcl-2, but had no change on total P38 MAPK. As expected, increased p-P38 MAPK and Bax were rescued by GAS in a dose-dependent manner. Meanwhile, GAS was able to increase Bcl-2 expression.

DISCUSSION

The present study revealed that GAS treatment effectively attenuated BCCAO-induced cognitive deficits, reduced hippocampus CA1 and CA3 pyramidal neuron morphological damage. Molecular biology analysis revealed that its underlying mechanism was mediated, at least in part, by attenuating deposition of Aβ, reducing BCCAO-enhanced autophagy, and suppressing neurons apoptosis. These novel findings provide pharmacological basis of GAS for VD.

Our previous studies found that rats subjected to BCCAO showed a significant decrease in hippocampus-dependent cognitive impairment (Li et al., 2015). Consistent with this observation, the present results of MWM demonstrated that significant learning and memory impairments appeared in BCCAO rats. However, long-term treatment with GAS significantly arrested the cognitive impairment. Furthermore,
HE staining revealed that morphological defects in the CA1 and CA3 areas of hippocampus were also founded, and GAS treatment ameliorated morphological damage in the CA1 and CA3 region, which was consistent with the behavioral results of the MWM. These results clearly demonstrate that GAS has beneficial effects on the cognitive impairment in rats with BCCAO.

A great quantity of Aβ deposition has been reported in BCCAO rats, and the degree of cognitive impairment is positively correlated with the expression of Aβ (Li et al., 2015). Thus, the strategies of restrain Aβ production and/or promote Aβ clearance can reduce the levels of Aβ, and can set-back BCCAO-induced cognitive impairment (Choi et al., 2011; Song et al., 2013; Han et al., 2015; Reijmer et al., 2016; Cai et al., 2017). Moreover, Aβ1−40 and Aβ1−42 are the two most pre-dominant forms of Aβ in the plaques. Previous research has suggested that GAS treatment markedly reduced the level of Aβ1−40 and Aβ1−42 in the hippocampus were detected. Similar to prior researches, the contents of Aβ1−40 and Aβ1−42 were significantly increased after BCCAO. However, GAS-treated BCCAO rats shown a reduction in Aβ1−40 and Aβ1−42 level. Therefore, one of the molecular mechanisms by which GAS attenuates BCCAO-induced cognitive deficits is related to elimination of Aβ.

As is known that, APP, the raw material of generation of Aβ, is processed along two alternative pathways: one is amyloidogenic pathway, in which β- and γ-secretases lead to the accumulation of Aβ, activation of β-secretase could generate neurotoxic Aβ; the other pathways is non-amyloidogenic pathway, which α- and γ-secretases lead to the production of soluble amyloid precursor protein-α (sAPPα). It is completely different from the amyloidogenic pathway, and the production of non-amyloidogenic pathway, sAPPα, has neurotropic and neuroprotective properties (Meineck et al., 2016). There is wide consensus that activation of α-secretase could increase production of neuroprotective sAPPα (Zhang et al., 2012; Corrigan et al., 2012a,b; Jeong, 2017; Yuksel et al., 2017).
FIGURE 6 | Effects of GAS on autophagy-related protein expression in the hippocampus. (A) Beclin-1, LC3-II, and p62 protein expression in the hippocampus. (B–D) Quantitation of Beclin-1, LC3-II, and p62 levels, respectively. Values are mean ± SD (n = 3). *P < 0.05; **P < 0.01 vs. sham; #P < 0.05; ##P < 0.01 vs. BCCAO.

FIGURE 7 | Effects of GAS on apoptosis-related protein expression in the hippocampus. (A) P38 MAPK, Bax, and Bcl-2 protein expression and P38 MAPK phosphorylation in the hippocampus. (B–D) Quantitation of p-P38 MAPK, P38 MAPK, Bax, and Bcl-2 levels, respectively. Values are mean ± SD (n = 3). *P < 0.05; **P < 0.01 vs. sham; #P < 0.05; ##P < 0.01 vs. BCCAO.
In productive process of Aβ, BACE1 is responsible for the chief function of β-secretase (Maloney and Lahiri, 2011). Once BACE1 unregulated, APP is processed along the amyloidogenic pathway, and resulted in the overexpression of neurotoxic Aβ (Zhu et al., 2013). ADAM10, a competitor of BACE1, is not only promoting the expression of α-secretase that combats formation of amyloidosis, but also enhancing release of neuroprotective sAPPα. Furthermore, in clean-up process of Aβ, IDE is one of the major proteases responsible for Aβ clearance enzyme in the hippocampal lysates and for the degradation of Aβ in cerebrospinal fluid and cytoplasm (Stargardt et al., 2013; Kukreja et al., 2014). Interestingly, in this study, we found that GAS not only inhibited Aβ production, but also promoted Aβ clearance following BCCAO in rats. Altogether, GAS downregulated BACE1, indirectly decreased Aβ neurotoxic injury, which reconfirm previous findings (Zhang J.S. et al., 2016). The findings that GAS upregulated ADAM10 and IDE expression are novel, and may be important against Aβ etiology.

The aggregation of Aβ after BCCAO is also correlated with abnormal autophagy. Aβ mediated autophagy flux and accumulation of autophagosomes; meanwhile, Aβ deposition was found to induce neuron apoptosis (Ghavami et al., 2014). Thus, novel agents targeting apoptosis inhibition and/or autophagy regulation might be potential neuroprotective drugs for VD. Previous studies have suggested that GAS treatment regulates autophagy and apoptosis dysfunction in astrocytes exposed to LPS in vitro (Wang et al., 2016), however, the effect of GAS on VD has not been reported. Here, we focused on the expression of autophagy and apoptosis mediators implicated in VD pathology. Autophagy, a double-edged sword, can be activated in different stress responses and in many pathological processes of diseases, which is thought to be a protective mechanism. Nevertheless, excessive autophagic activity may lead to a collapse of cellular functions. In this study, we detected some markers of autophagy, such as Beclin-1, LC3-II, and p62. Beclin-1 is an important autophagy-regulatory gene that reflects autophagic activity (Shin et al., 2014). Besides, LC3-II embedded on the membrane of autophagic vacuole, the content of which represents autophagy activity. p62, an autophagy regulatory factor, overexpression of p62 promotes the degradations of abnormal proteins (Xu et al., 2014). Consistent with previous findings, we found that Beclin-1, LC3-II, and p62 were increased after BCCAO (Gao et al., 2015; Zou et al., 2017). Fortunately, GAS treatment markedly decreased the expression of Beclin-1, LC3-II, and p62, indicating BCCAO activated autophagic flux, and GAS repressed BCCAO-induced autophagic flux. Moreover, we found autophagy suppression may not be the unique mechanism of the protective effect of GAS on BCCAO-induced injury, and apoptosis inhibition may also be involved. Apoptosis plays an important role in the process of neuronal death after BCCAO (Zhao et al., 2018). As a result of GAS treatment, a marked reduction in the number of apoptotic cells in the hippocampus was observed (Figure 3). Further study indicated that GAS against apoptosis via enhancing Bcl-2 expression and reducing Bax expression. It is worth mentioning that, Bcl-2 is not only an anti-apoptotic protein, but also an anti-autophagy protein via interaction with Beclin-1 (Mukhopadhyay et al., 2014). Thus, we therefore postulated that the protective effect of GAS on VD is associated with upregulation of Bcl-2, and then combined with excessive Beclin-1 to block autophagy. These findings indicated that attenuation of excessive autophagy and apoptosis is important molecular mechanisms of GAS against VD.

The MAPK consists of three well-defined subgroups, among which P38 MAPK is closely associated with autophagy and apoptosis (Ghatan et al., 2000; Jiang et al., 2013; Han et al., 2014; Xue et al., 2015). Coincidentally, GAS showed inhibitory effect on P38 MAPK phosphorylation in previous studies (Yang et al., 2013; Jiang et al., 2014), thus we assessed the phosphorylation of P38 MAPK. As expected, treatment with GAS inhibited phosphorylation of P38 MAPK. Thus, we speculate that GAS blocks P38 MAPK activation, and consequently suppress excessive autophagy and apoptosis. Regrettfully, downstream of P38 MAPK signaling pathway, related to autophagy and apoptosis, has not been rigorously studied in this study, and selective P38 MAPK antagonists or P38 MAPK knock-out models will be further required to clarify the exact molecular mechanism. Thus, the beneficial effects of GAS also involve its modulation on MAPK signaling pathway.

CONCLUSION

We demonstrated that GAS attenuates BCCAO-induced cognitive deficits and hippocampus neuron damage, and its underlying mechanism is likely due to decreasing Aβ deposition, retraining excessive autophagy and apoptosis, and regulating P38 MAPK signaling pathway.

AUTHOR CONTRIBUTIONS

J-SS conceived and designed all the experiments. BL, J-MG, and FL performed the experiments. FL and Q-HG finished the data analysis. BL, J-MG, FL, and Q-HG wrote and revised the manuscript. All the authors reviewed the manuscript and approved the submitted manuscript.

FUNDING

The present study was supported by the Brainstorm Project on Social Development by Department of Science and Technology of Guizhou Province [Grant No. JZ (2014) 2015].

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2018.00405/full#supplementary-material

FIGURE S1 | Chemical structure of gastrodin.
Jeong, S. (2017). Molecular and cellular basis of neurodegeneration in Alzheimer's disease. *Brain Res.* 1451, 87–99. doi: 10.1016/j.brainsci.2012.02.045

Choi, B. R., Lee, S. B., Han, J. S., Woo, S. K., Kim, K. M., Choi, D. H., et al. (2011). Ischemic preconditioning mediates neuroprotection against ischemia in mouse hippocampal CA1 Neurons by inducing autophagy. *PLoS One* 10:e0137146. doi: 10.1371/journal.pone.0137146

Gao, C., Cai, Y., Zhang, X., Huang, H., Wang, J., Wang, Y., et al. (2015). Gastrodin protects against MPP+-induced oxidative stress by up-regulates heme oxygenase-1 expression in rat nigrostriatal dopaminergic neurons. *Acta Pharmacol. Sin.* 36, 40–54. doi: 10.1038/jcbs.2015.233

Liu, L., Gao, N., Xin, W., Lu, X., Shi, S., et al. (2016). Effect of hydrogen gas on the survival rate of mice following global cerebral ischemia. *Shock* 37, 645–652. doi: 10.1097/SHK.0000000000000424

Nagatani, K., Wada, K., Takeuchi, S., Kobayashi, H., Uozumi, Y., Otani, N., et al. (2012). Effect of hydrogen gas on the survival rate of mice following global cerebral ischemia. *Shock* 37, 645–652. doi: 10.1097/SHK.0b013e31824ed57c

Reijmer, Y. D., van Veluw, S. J., and Greenberg, S. M. (2016). Ischemic brain injury in cerebral amyloid angiopathy. *J. Cereb. Blood Flow Metab.* 36, 40–54. doi: 10.1177/0271678X15606758

Schutte, B., Nuydens, R., Geerts, H., and Ramaker, K. (1998). Annexin V binding assay as a tool to measure apoptosis in differentiated neuronal cells. *J. Neurosci.* Methods. 86, 63–69. doi: 10.1016/S0165-0270(98)00147-2

Shin, J. Y., Park, H. J., Kim, H. N., Oh, S. H., Bae, J. S., Ha, H. J., et al. (2012). Effect of hydrogen gas on the survival rate of mice following global cerebral ischemia. *Shock* 37, 645–652. doi: 10.1097/SHK.0b013e31824ed57c

Smith, E. E. (2017). Clinical presentations and epidemiology of vascular dementia. *Clin. Sci.* 111, 1059–1068. doi: 10.1042/CS20160607

Song, B., Ao, Q., Niu, Y., Shen, Q., Zuo, H., Zhang, X., et al. (2013). Amyloid beta-peptide worsens cognitive impairment following cerebral ischemia-reperfusion injury. *Neural Regen. Res.* 8, 2449–2457. doi: 10.3969/j.issn.1673-5374.2013.26.006

Stargardt, A., Gillis, J., Kanphusui, W., Wiemhofer, A., Kooijman, L., Raspe, M., et al. (2013). Reduced amyloid-beta degradation in early Alzheimer’s disease but not in the APPSwed1E9 and 3xTg-AD mouse models. *Aging Cell* 12, 499–507. doi: 10.1111/acel.12074

Wang, X. S., Tian, Z., Zhang, N., Han, J., Guo, H. L., Zhao, M. g., et al. (2016). Protective effects of gastrin against autophagy-mediated astrocyte death. *Phytother. Res.* 30, 386–396. doi: 10.1002/ptr.5538

Won, J. S., Kim, J., Annamalai, B., Shumugavel, A., Singh, I., and Singh, A. K. (2013). Protective role of S-nitrosoglutathione (GSNO) against cognitive impairment in rat model of chronic cerebral hyperperfusion. *J. Alzheimers Dis.* 34, 621–635. doi: 10.3233/JAD-121786

Xiong, Z., Lu, W., Zhu, L., Zeng, L., Shi, C., Jing, Z., et al. (2017). D3-n-Butylphthalide treatment enhances hemodynamics and ameliorates memory impairment in a rat model of chronic cerebral ischemia. *Neural Regen. Res.* 12, 499–507. doi: 10.1111/acel.12074
deficits in rats with chronic cerebral hypoperfusion. *Front. Aging Neurosci.* 9:238. doi: 10.3389/fnagi.2017.00238

Xu, Y., Zhang, J., Tian, C., Ren, K., Yan, Y. E., Wang, K., et al. (2014). Overexpression of g62/SQSTM1 promotes the degradations of abnormally accumulated PrP mutants in cytoplasm and relieves the associated cytotoxicities via autophagy-lysosome-dependent way. *Med. Microbiol. Immunol.* 203, 73–84. doi: 10.1007/s00430-013-0316-z

Xue, Q., Wang, X., Wang, P., Zhang, K., and Liu, Q. (2015). Role of p38MAPK in apoptosis and autophagy responses to photodynamic therapy with Chlorin e6. *Photodiagnosis Photodyn. Ther.* 12, 84–91. doi: 10.1016/j.pdpdt.2014.12.001

Yang, P., Han, Y., Gui, L., Sun, J., Chen, Y. L., Song, R., et al. (2013). Gastrodin attenuation of the inflammatory response in H9c2 cardiomyocytes involves inhibition of NF-kappaB and MAPKs activation via the phosphatidylinositol 3-kinase signaling. *Biochem. Pharmacol.* 85, 1124–1133. doi: 10.1016/j.bcp.2013.01.020

Yuksel, M., Biberoglu, K., Onder, S., Akbulut, K. G., and Tacal, O. (2017). Effects of phenothiazine-structured compounds on APP processing in Alzheimer's disease cellular model. *Biochimie* 138, 82–89. doi: 10.1016/j.biochi.2017.04.012

Zhang, D., Xiao, Y., Lv, P., Teng, Z., Dong, Y., Qi, Q., et al. (2018). Edaravone attenuates oxidative stress induced by chronic cerebral hypoperfusion injury: role of ERK/Nrf2/HO-1 signaling pathway. *Neurol. Res.* 40, 1–10. doi: 10.1080/01616412.2017.1376457

Zhang, H., Ma, Q., Zhang, Y. W., and Xu, H. (2012). Proteolytic processing of Alzheimer's beta-amyloid precursor protein. *J. Neurochem.* 120(Suppl. 1), 9–21. doi: 10.1111/j.1471-4159.2011.07519.x

Zhang, J. S., Zhou, S. F., Wang, Q., Guo, J. N., Liang, H. M., Deng, J. B., et al. (2016). Gastrodin suppresses BACE1 expression under oxidative stress condition via inhibition of the PKR/eIF2alpha pathway in Alzheimer's disease. *Neuroscience* 325, 1–9. doi: 10.1016/j.neuroscience.2016.03.024

Zhang, Y., Wang, L.-L., Wu, Y., Wang, N., Wang, S.-M., Zhang, B., et al. (2016). Paeoniflorin attenuates hippocampal damage in a rat model of vascular dementia. *Exp. Ther. Med.* 12, 3729–3734. doi: 10.3892/etm.2016.3849

Zhang, R., Peng, Z., Wang, H., Xue, F., Chen, Y., Wang, Y., et al. (2014). Gastrodin ameliorates depressive-like behaviors and up-regulates the expression of BDNF in the hippocampus and hippocampal-derived astrocyte of rats. *Neurochem. Res.* 39, 172–179. doi: 10.1007/s11064-013-1203-0

Zhao, T., Fu, Y., Sun, H., and Liu, X. (2018). Ligustrazine suppresses neuron apoptosis via the Bax/Bcl-2 and caspase-3 pathway in PC12 cells and in rats with vascular dementia. *IUBMB Life* 70, 60–70. doi: 10.1002/iub.1704

Zhu, Y., Zhang, Q., Zhang, W., Li, N., Dai, Y., Tu, J., et al. (2017). Protective effect of 17beta-estradiol upon hippocampal spine density and cognitive function in an animal model of vascular dementia. *Sci. Rep.* 7:42660. doi: 10.1038/srep42660

Zhu, Z., Yan, J., Jiang, W., Yao, X. G., Chen, J., Chen, L., et al. (2013). Arctigenin effectively ameliorates memory impairment in Alzheimer's disease model mice targeting both beta-amyloid production and clearance. *J. Neurosci.* 33, 13138–13149. doi: 10.1523/JNEUROSCI.4790-12.2013

Zou, W., Song, Y., Li, Y., Du, Y., Zhang, X., and Fu, J. (2017). The role of autophagy in the correlation between neuron damage and cognitive impairment in rat chronic cerebral hypoperfusion. *Mol. Neurobiol.* 55, 776–791. doi: 10.1007/s12035-016-0351-z

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Liu, Gao, Li, Gong and Shi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.