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Therapeutic effect of *Ginkgo biloba* polysaccharide in rats with focal cerebral ischemia/reperfusion (I/R) injury

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ABSTRACT

An araban type polysaccharide (GBPw) was purified from the leaves of *Ginkgo biloba*. The present study aimed to investigate the protective effects of GBPw on focal ischemia/reperfusion (I/R) injury in rat brain. The results of this study demonstrated that GBPw had a positive effect on the rat brain when administered 7 days before focal cerebral I/R injury. This effect was evident with the improvements in neurological deficits, reduction in infarct volume, MDA content and the levels of pro-inflammatory cytokines (TNF-α and IL-1β), and elevation in the SOD and MPO activities and the levels of anti-inflammatory cytokine (IL-10). Thus, the beneficial effects of GBPw on cerebral I/R injury may result from the reduction of oxidative stress and the inhibition of NO production and inflammation induced by I/R. The neuroprotective effects of GBPw supplement may have potential implication in the future for prevention/protection against cerebral ischemic stroke.

1. Introduction

Acute ischemic stroke is one of the leading causes of adult disability and death in the world, and the majority of ischemic strokes results from occlusion of the middle cerebral artery, which leads to a reduction of cerebral blood flow in a specific region. Reperfusion of the tissue is associated with inflammation, increased reactive oxygen species, necrosis and apoptosis. Hence, damage to the brain will continue even after the blood flow is restored. Reperfusion after ischemia leads to profound cerebral microcirculatory disturbance and even death of neurons (Aronowski, Strong, & Grotta, 1997), thus protecting the brain from reperfusion injury after ischemia is an alternative for therapy of stroke. Although numerous interventions have been investigated, few neuroprotective agents have been successfully translated from basic research into clinical applications (Linares & Mayer, 2009). Thrombolytic therapy and intravascular techniques are recommended to restore blood flow to the brain in acute ischemic stroke but carries with the risk of hemorrhage and aggravates cerebral damage caused by reperfusion (Aronowski et al., 1997).

Over the past decade, intense interest has focused on discovering effective agents from medicinal plants, especially traditional Chinese herbal medicines (CHM), for the prevention and treatment of ischemic stroke, since many CHMs are reported to possess cardioprotective or neuroprotective activities (He et al., 2012; Huang, Tan, Chen, & Deng, 2012; Sun et al., 2010). Therefore, a search for safe and effective agents from CHMs for management of ischemic brain injury is urgently needed.

The extract of *Ginkgo biloba* L. (Ginkgoaceae) has been extensively used in a variety of cardiovascular and cerebrovascular diseases such as ischemia, dementia, and depression (Ahlemeyer & Krieglstein, 2003; Gertz & Kiefer, 2004; Nishida & Satoh, 2004; Saleem, Zhuang, Biswal, Christen, & Dore, 2008; Sierpina, Wollschlaeger, & Blumenthal, 2003). Although highly neuroprotective effects of *G. biloba* extract against ischemia/reperfusion (I/R) injury has been well proven (Krieglstein et al., 1995; Wang, Wang, Zhao, & Zhang, 1998), the mechanism and mode underlying the therapeutic effects is still under investigation. It has been revealed that neuroprotection action of *G. biloba* extract during cerebral I/R injury might be related to two major groups, namely flavonols and lactones (Cho et al., 2009; Dubber & Kanfer, 2004), while nothing is known about the neuroprotective effect of purified water-soluble *G. biloba* polysaccharide on I/R-induced cerebral injury. Therefore the present study was undertaken to purify the polysaccharide from this plant and investigate its neuroprotective potential and mechanism in the middle

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cerebral artery occlusion (MCAO)-induced focal cerebral I/R model in rats.

2. Materials and methods

2.1. Materials and chemicals

The leaves of *G. biloba* L were purchased from Hebei Anguo Medicinal Materials Market, China, and identified by Dr. Weiqi Zhong at Northeast Agricultural University. Sephadex G-200 and DEAE-52 cellulose were from Pharmacia (Sweden). Standard dextran was from Sigma. All other reagents were of the highest available quality in China.

2.2. Isolation and purification of polysaccharide GBPw

The dried leaves (700 g) of *G. biloba* were soaked with 95% EtOH for 6 h to remove lipids and pigment, followed by filtration. The residue was dried in air and then extracted with boiling water for three times (6 h for each). After cooling, the supernatant was separated from the residues by centrifugation (2000 × *g*, 20 min). The supernatants was deproteined by the method of Sevag (Staub, 1965), concentrated under reduced pressure and dialyzed against distilled water at 30 °C for three successive days to exclude most of low molecular weight compounds with a dialysis bag (MWCO 5000, Sigma). The retained fraction was recovered, concentrated under reduced pressure, and precipitated by addition of 4-fold volume of 95% (v/v) ethanol at 4 °C overnight. The precipitated polysaccharides were collected by centrifugation (2000 × *g*, 20 min) and lyophilized, and 7.6 g of crude polysaccharides (CGBP) were obtained.

CGBP (1.0 g) was dissolved in distilled water, centrifuged, and then the supernatant was loaded onto a column (26 mm × 300 mm) of DEAE-52 cellulose column equilibrated with distilled water. After loading with sample, the column was eluted with distilled water, followed by 0.1, 0.2, 0.4 and 1.0 M NaCl, sequentially, at a flow rate of 1.0 mL/min. The fraction containing polysaccharide eluted by deionized water was further applied to a column (2.5 cm × 100 cm) of Sephadex G-200 (26 mm × 800 mm) and eluted with 0.1 M NaCl at a flow rate of 1 mL/min. The eluate from each collecting tuber was examined for the presence of carbohydrate using the phenol–sulfuric acid method. The fractions with rose color were combined, concentrated under vacuum, dialyzed with membrane (MWCO: 1 k) to desalinate and lyophilized to afford a major purified polysaccharide (GBPw). The GBPw was kept in dryer for further analysis.

2.3. Chemical properties of GBPw

Total carbohydrate and protein of the purified polysaccharide were determined by the phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and Bradford (1976), respectively. Uronic acid content was determined according to a meta-hydroxydiphenyl colorimetric method (Filisetti-Cozzi & Carpita, 1991). The molecular weight was determined by high-performance gel-permeation chromatography (HPGPC) (Yamamoto, Nunome, Yamauchi, Kato, & Sone, 1995) with a Waters HPLC system, and dextran standards with different molecular weights of standard T-series Dextran (T-2000, T-580, T-190, T-70, T-10, Sigma) were used for preparing calibration curve. The monosaccharide compositions were analyzed by gas chromatography (GC, Agilent USA) with a flame-ionization detector. The polysaccharides (10 mg) were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100 °C for 8 h, followed by evaporation to dryness and successive reduction with $NaBH_4$ and acetylation with Ac_2O–pyridine at room temperature for 12 h. The Ac_2O was destroyed with ice-water, and the resulting alditol acetates extracted with $CHCl_3$ and analyzed by GC as described by Santos-Neves et al. (2008).

2.4. Animals

Male Sprague-Dawley rats, weighing 280 ± 20 g, were purchased from the Experimental Animal Center of Harbin Medical College, Harbin, China). The rats were kept at a constant room temperature of 22 °C under 12 h light–dark cycle and free access to tap water and standard food. All animal experiments were performed in accordance with the guidelines approved by the Animal Ethics Committee of Harbin Medical College.

2.5. Preparation of middle cerebral artery occlusion (MCAO) model

After 7 days of pretreatment with GBPw, rats were subjected to a middle cerebral artery occlusion (MCAO) according to the method of Hossmann and Kogune (Wang, 1998). Briefly, 1% pentobarbital sodium solution (30 mg/kg) was used with an intraperitoneal (i.p.) injection to the rats. Under sterile conditions, the right common artery, external carotid artery and internal carotid artery were exposed, respectively. A 4–0 nylon filament thread with a blunted tip was inserted into the internal carotid artery and advanced into the anterior cerebral artery to occlude the middle cerebral artery (MCA). After 60 min of MCAO, the nylon filament was taken out and the blood was reflowed for 24 h. In sham-operated rats, the carotid arteries were surgically exposed for insertion of the filament, but no filament was inserted. During surgery, the body temperature was monitored with a rectal thermistor and maintained at 37 ± 0.5 °C using a lamp.

2.6. Experimental protocol

To investigate the neuroprotective effects of GBPw, we used the rat MCAO model. A total of 60 rats were randomly divided into five groups: sham operation group (Sham group), cerebral IR group (Model group) and GBPw treatment group at three doses of 100, 200 and 400 mg/kg body weight (GBPwL, GBPwM and GBPwH group). GBPw (100, 200 and 400 mg/kg) was dissolved in saline and orally administered once a day for 7 days prior to reperfusion. The sham group and IR group rats were administered the same dose of sterile saline using the same time schedule used in the GBPw treated group. The rats were sacrificed after 24 h of reperfusion. The blood samples were collected and the brains were harvested. Of the 12 rats in each group, 6 rats were used for assessment of neurobehavioral deficit and infarct volume and the other 6 rats were used for determination of malondialdehyde (MDA), NO and cytokines (TNF-α, IL-1β, and IL-10) levels and superoxide dismutase (SOD) and myeloperoxidase (MPO) activities in brain tissue.

2.7. Neurologic evaluation

Neurological deficits in the vehicle- and drug-pretreated group were determined after 24 h of reperfusion (Longa, Weinstein, Carlson, & Cummins, 1989). The neurological findings were scored on a 5-point scale in which rats with no neurologic deficit scaled 0, rats with (failure to extend left forepaw fully) a mild focal neurologic deficit scaled 1, rats with (circling to the left) a moderate focal neurologic deficit scaled 2, rats with (falling to the left) a severe focal deficit scaled 3, rats with a score of 4 did not crawl spontaneously and was unconscious. The observer had no knowledge of which treatment had been administered. The above procedures were performed in a blinded manner.

Table 1
General chemical properties and molecular weight of GBPw.

Sample	Neutral sugar (mol%)				Total carbohydrate (wt%)	Uronic acid (wt%)	Protein (wt%)	Molecular weight (kDa)
	Arabinose	Mannose	Galactose	Glucose				
GBPw	8.2	0.5	0.5	0.3	92.1	–	4.4	28

2.8. Evaluation of cerebral infarction by TTC staining

Infarct volume in the brain was evaluated using the TTC staining method (Ghisalberti, 1997). Briefly, after 24 h of postischemic reperfusion, brains was quickly removed and sliced into 2-mm thickness. Coronal slices were immersed into a solution of 2,3,7-triphenyltetrazolium chloride (2% TTC) for 10 min at 37 °C, and then fixed by immersion in 4% paraformaldehyde at 4 °C overnight (Bederson et al., 1986). Infarct area was quantified with the professional image analysis software. Infarct volumes of each slice were calculated by multiplying the infarct area of the slice by its thickness (2 mm) and the data were expressed as % of the infarct volume to total volume of the brain.

2.9. Determination the content of MDA and NO, and the activities of SOD and myeloperoxidase (MPO) in brain tissues of cerebral I/R injured rats

After MCAO under anesthesia, the right hemisphere of the brain was homogenated and MDA and NO levels and SOD activity in the supernatant of cerebral tissues were determined by thiobarbituric acid method (Ohkawa, Ohishi, & Yagi, 1979), Griess method (Szabo, Southan, Wood, Thiemermann, & Vane, 1994) and xanthine oxidase method (Nandi & Chatterjee, 1988), respectively. MPO activity was measured with ELISA kit (JianChengShengWu, Nan Jing, China) according to the manufacturers' instruction.

2.10. Measurement of cytokines in brain tissues of cerebral I/R injured rats

TNF-α in 10% cerebral supernatant was measured by RIA detection kit ((JianChengShengWu, Nan Jing, China) according to the manufacturers' instruction. TNF-α concentration was determined according to a standard curve and then normalized by protein concentration. IL-1β and IL-10 were measured using commercially available quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (JianChengShengWu, Nan Jing, China). Measurements were conducted with a plate reader at a wavelength of 450 nm. IL-1β and IL-10 concentrations was determined according to a standard curve and then normalized by protein concentration.

2.11. Statistical analysis

All data are presented as mean ± SD. Statistical differences between the experimental groups were analyzed by using the one-way ANOVA. T test was performed and *P* value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Isolation and characterization of GBPw

The water-soluble crude polysaccharides (CGBP) with a yield of 10.85% (w/w) of starting material were obtained from the leaves of *G. biloba* by defatting with ethanol, hot water extraction, ethanol precipitation, deprotein by Sevag method, dialysis against water and drying in freeze dryer. Then CGBP was subjected to a DEAE–cellulose chromatography and eluted with distilled water, followed by 0.1, 0.2, 0.4 and 1.0 M NaCl at a flow rate of 1.0 mL/min. A 0.2 mL sample from each collected fraction (8 mL) was analyzed for carbohydrate content by a phenol–sulfuric acid assay. Among them, neutral fraction CGBPw showed the relatively high content. Therefore CGBPw was repeatedly subjected to size-exclusion column chromatography on a Sephadex G-200 gel filtration column and eluted with 0.1 M NaCl at a flow rate of 1 mL/min. Fractions containing one major polysaccharide peak were collected and then freeze-dried to yield GBPw (2.17 g, 0.31% of starting material).

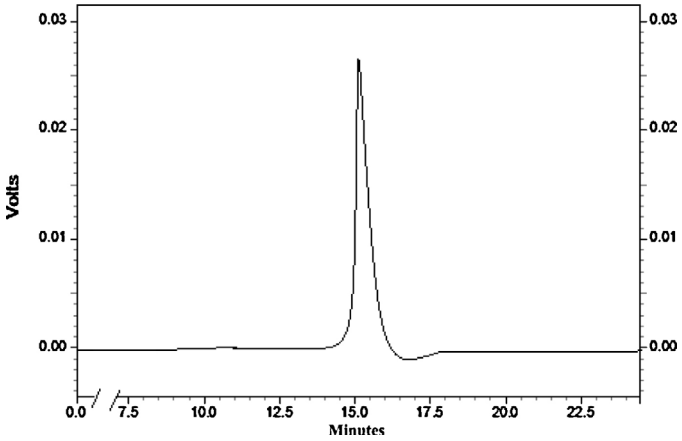

Fig. 1. HPGPC profile of GBPw.

The total carbohydrate content, uronic acid and protein of GBPw were 92.1%, 0.0%, and 4.4%, as determined by phenol–sulfuric acid method, m-hydroxydiphenyl colorimetric method and Bradford method, respectively. The HPGPC (Fig. 1) profile of GBPw showed a single and nearly symmetrical signal, indicating that GBPw was a homogeneous polysaccharide. According to the retention time, the average molecular weight (Mw) of GBPw was approximately 28 kDa. Sugar analysis revealed GBPw consisted of four kinds of monosaccharide: arabinose, mannose, galactose and glucose, whose molar ratios were 8.2:0.5:0.5:0.3, respectively (Table 1). It was clear that the predominantly composition monosaccharide in the polysaccharide were arabinose up to 86.31% (mol.%) of total carbohydrates.

3.2. Effect of GBPw on infarct volume in cerebral I/R injured rats

TTC staining of coronal sections was used to measure cerebral infarction (Table 2). The percentage of the cerebral infarct volume

Table 2
The effect of GBPw on the infarct volume in the brain and neurological deficit score in rats with cerebral I/R injury.

Group	Infarct volume (%)	Neurological deficit score
Sham group	0.2 ± 0.0	0.0 ± 0.0
Model group	35.7 ± 3.1$^{\#\#\#}$	3.3 ± 0.6$^{\#\#}$
GBPwL	28.4 ± 2.6*	2.3 ± 0.5*
GBPwM	26.7 ± 2.7**	1.9 ± 0.7**
GBPwH	24.4 ± 2.8**	1.7 ± 0.6**

Data are mean ± SD values ($n = 6$). GBPwL, GBPwM and GBPwH, rats pretreated with GBPw at 100, 200, or 400 mg/kg before MCAO, respectively. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus model group.

Table 3
Effects of GBPw on the content of MDA and NO, and the activities of SOD and myeloperoxidase (MPO) in the brain of rats with cerebral I/R injury.

Group	MDA (nmol/mg protein)	NO (μmol/L)	SOD (U/mg protein)	MPO (U/g wet tissue)
Sham group	1.81 ± 0.33	106.58 ± 26.60	63.85 ± 7.21	0.07 ± 0.02
Model group	3.45 ± 0.52$^{\#\#}$	253.54 ± 42.75$^{\#\#}$	43.64 ± 6.03$^{\#\#}$	0.25 ± 0.07$^{\#\#}$
GBPwL	2.34 ± 0.36**	163.57 ± 30.01*	52.87 ± 6.21**	0.15 ± 0.06*
GBPwM	2.15 ± 0.28**	135.85 ± 26.13**	54.60 ± 5.66**	0.10 ± 0.05**
GBPwH	2.07 ± 0.23**	126.84 ± 22.35**	61.09 ± 7.22**	0.08 ± 0.05**

Data are mean ± SD values (n = 6). GBPwL, GBPwM and GBPwH, rats pretreated with GBPw at 100, 200, or 400 mg/kg before MCAO, respectively. $^{\#\#}P < 0.01$ versus sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus model group.

Table 4
Effects of GBPw on the content of cytokine concentrations in the brain of rats with cerebral I/R injury.

Group	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)	IL-10 (ng/mg protein)
Sham group	1.02 ± 0.21	1.78 ± 0.35	1.81 ± 0.27
Model group	3.05 ± 0.49$^{\#\#}$	5.64 ± 0.79$^{\#\#}$	0.42 ± 0.13$^{\#\#}$
GBPwL	2.22 ± 0.31*	3.40 ± 0.62**	0.65 ± 0.12
GBPwM	1.54 ± 0.26**	2.58 ± 0.41**	0.83 ± 0.18**
GBPwH	1.15 ± 0.24**	2.05 ± 0.22**	1.50 ± 0.21**

Data are mean ± SD values (n = 6). GBPwL, GBPwM and GBPwH, rats pretreated with GBPw at 100, 200, or 400 mg/kg before MCAO, respectively. $^{\#\#}P < 0.01$ versus sham group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus model group.

was 0.2 ± 0.0% in the sham group and 35.7 ± 3.1% in the model group. Pretreatment with GBPw significantly reduced the volume of infarction to 28.4 ± 2.6 at the dose of 100 mg/kg, 26.7 ± 2.7 at the dose of 200 mg/kg, and 24.4 ± 2.8 at the dose of 400 mg/kg, respectively, compared to the IR control group ($P < 0.05$ or 0.01).

3.3. Effect of GBPw on neurological deficit in cerebral I/R injured rats

Twenty-four hours after the onset of MCAO, neurological deficit score in the model group was significantly higher than that of the sham group ($P < 0.001$), indicating that the MCAO model had effectively established cerebral ischemia. Pretreatment with GBPw induced a dose-related amelioration of the neurological deterioration ($P < 0.05$ or 0.01) as compared to the model control group (Table 2). The neurological deficit scores were positively correlated with the infarct volume of the brain in different groups of rats.

3.4. Effect of GBPw on the content of MDA and NO, and the activities of SOD and myeloperoxidase (MPO) in brain tissues of cerebral I/R injured rats

The brain SOD activity was significantly decreased in the model group ($P < 0.01$) compared with the sham group; whereas the content of MDA and NO were increased ($P < 0.01$). However, treatment with GBPw markedly inhibited the attenuation of SOD activity and the increased MDA and NO content in brain tissue (Table 3).

The accumulation of neutrophils in the ischemic area was investigated by measuring MPO activity in the brain tissue. As shown in Table 3, MPO activity (0.25 ± 0.07 U/g wet tissue) was significantly higher ($P < 0.01$) in the brain tissue of model control rats as compared to the values (0.07 ± 0.02 U/g wet tissue) in the sham-operated group. In contrast, pretreatment with GBPw (100, 200 and 400 mg/kg) reduced MPO activity from 0.25 ± 0.07 U/g wet tissue to 0.15 ± 0.06, 0.10 ± 0.05 and 0.08 ± 0.05, respectively.

3.5. Effect of GBPw on the content of cytokines TNF-α, IL-1β, and IL-10 in brain tissues of cerebral I/R injured rats

Concentrations of pro-inflammatory cytokines (TNF-α and IL-1β) in cerebral tissue of vehicle-pretreated ischemic rats were significantly higher as compared with those of sham-operated control rats ($P < 0.01$). Treatment with GBPw at 100, 200 and 400 mg/kg prior to MCAO performance resulted in significant decrease in TNF-α and IL-1β concentrations as compared with vehicle treatment ($P < 0.01$). With regard to anti-inflammatory cytokine IL-10, a noticeable increase in cerebral secretion of IL-10 was observed in GBPw-pretreated group as compared to the model group ($P < 0.01$). However, the cerebral IL-10 level (0.42 ± 0.13 pg/mg protein) of rats that had undergone cerebral I/R injury exhibited a 77% decrease as compared with that (1.81 ± 0.27 pg/mg protein) of the sham-operated animals. I/R induced decrease in the IL-10 secretion of brain tissue was reversed by GBPw at doses of 200 and 400 mg/kg compared with the model control ($P < 0.01$), as a result of attenuation of inflammation injury to brain (Table 4).

4. Conclusions

The MCAO model established by thread insertion is by far the most commonly used stroke model, the pathophysiology presented in which closely matches that in stroke patients suffered from focal cerebral arterial thrombosis (Longa et al., 1989). Using this model, pretreatment with GBPw was found to provide significant protection against the neuronal damage induced by cerebral I/R in rats. Our results showed clearly that a significant dose-dependent reduction of infracted volume, one most vigorous index of ischemic brain damage, was observed after GBPw administration in cerebral I/R rats. These changes were also reflected in the improvement of neurological deficit, which was evident from the decreased neurological deficit scores (Devries, Nelson, Traystman, & Hurn, 2001).

There is evidence that excess reactive oxygen radicals (ROS) are produced and released in the process of cerebral I/R injury, and these highly reactive chemicals attack lipids, proteins and nucleic acids, leading to lipid peroxidization and impairment of cell functions (Evans, 1993; Schmidley, 1990). The level of MDA is one of the most sensitive indicators of lipid peroxidation (Gutteridge, 1995). Superoxide dismutase (SOD), one of free radical scavenging enzymes, has a crucial role in the resistance of the neuronal cells to ROS-induced cell death and serves as a crucial index of antioxidation (Fujimura et al., 2000; Mizuno, Umemura, & Nakashima, 1998; Satoh, Ikeda, Shioda, Tobe, & Yoshikawa, 2002). In the present study, the brain SOD activity was found to be significantly decreased and the content of MDA elevated in the vehicle treated group after I/R, suggesting the involvement of free radicals in neuronal injury. All the alterations induced by MCAO and reperfusion were significantly attenuated by pretreatment of GBPw (100, 200 and 400 mg/kg) 7 days prior to the induction of cerebral I/R injury. The results of MDA levels and SOD activity are consistent with

a reduction of cerebral infarction as well as the neurological and motor behavioral recovery. This suggests that the effectiveness of GBPw in focal ischemia most probably by virtue of its antioxidant property.

Excessive amounts of NO can cause metabolic deterioration of the ischemic penumbra and promote neuronal death with larger infarction (Matsui, Nagafuji, Kumanishi, & Asano, 1999). After brain ischemia, nitric oxide derived from neuronal NOS contributes to the early stages of ischemic brain damage (Iadecola & Ross, 1997; Zhang, Xu, & Iadecola, 1995). Moreover, nitric oxide promotes oxidative damage by reacting with superoxide anion to form the oxidant compound peroxynitrite with deleterious effects on neuronal survival (Beckman, Beckman, Chen, Marshall, & Freeman, 1990). The results showed that GBPw treatment significantly reduced NO overproduction in rats undergoing transient brain I/R injury, suggesting that the dropping NO level may be, at least partly, involved in the protective effects of GBPw against cerebral I/R injury.

Various proinflammatory cytokines, such as TNF-α and IL-1β, secreted excessively in stroke and play central roles in the exacerbation of brain damage following stroke (Hallenbeck, 2002; Rothwell, Allan, & Toulmond, 1997; Wang, Wu, Huang, & Yang, 2004). Findings of in vivo studies demonstrated that focal cerebral I/R injury stimulated the release of TNF-α and IL-1β in brain. This observation is consistent with the results of previous studies that these two pro-inflammatory cytokines might be involved in reperfusion injury after transient brain ischemia (Tang, Liu, Hsieh, & Hsieh, 2010). However, treatment with GBPw prior to imposition of I/R resulted in a marked decrease in cortical TNF-α and IL-1β concentrations. Interestingly, anti-inflammatory cytokine IL-10 concentration negatively correlates with TNF-α and IL-1β levels in brain, suggesting that GBPw treatment after focal cerebral I/R injury may promote IL-10's feedback regulatory activity in rats. In consistence with this, we observed that brain infarction following reperfusion was accompanied by a rise in cerebral MPO activity in vehicle treated MCAO rats. MPO is a reductase primarily existing in neutrophil, and traditionally described as a potential marker for neutrophil infiltration within damaged brain tissue (Barone et al., 1992). MPO activity in all GBPw-treated groups decreased which indicates the potent anti-inflammatory effect of GBPw on focal cerebral ischemia. This observation strongly supports an anti-inflammatory action of GBPw sufficient to protect against focal cerebral I/R injury.

In conclusion, treatment of rats with GBPw before focal I/R injury decreases cerebral infarct size and improve neurological deficits. The neuroprotective effects of GBPw are mediated by suppression of NO production, decreased concentrations of TNF-α and IL-1β, increased concentration of IL-10, and inhibition of oxidative stress as evidenced by increased SOD activity and decreased MDA level. Based on the anti-inflammatory and antioxidant effects of GBPw observed in the present report, GBPw possesses significant therapeutic potential for prevention or/and treatment of cerebral ischemic injury.

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