Carbohydrate Polymers 94 (2013) 731–736

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Extraction and chemical characterization of *Angelica sinensis* polysaccharides and its antioxidant activity

Songtao Ai[a], Xindong Fan[a], Linfeng Fan[a], Qi Sun[a], Yu Liu[a], Xiaofeng Tao[a,*], Kerong Dai[b,*]

[a] *Department of Radiology, Shanghai Ninth People's Hospital Affiliated Shanghai JiaoTong University, School of Medicine, Shanghai, 200011, China*
[b] *Engineering Research Center of Digital Medicine, Ministry of Education, Department of Orthopedics, Shanghai Ninth People's Hospital Affiliated Shanghai JiaoTong University, School of Medicine, Shanghai, 200011, China*

ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form 5 February 2013
Accepted 9 February 2013
Available online 18 February 2013

Keywords:
Angelica sinensis
Antioxidant
Rabbit
SOD
Extraction
Orthogonal array experimental design
CT perfusion imaging

ABSTRACT

In the present study, the *Angelica sinensis* polysaccharides (ASP) extraction procedure was optimized by an L_9 (3^4) orthogonal array experimental design (OAD) with four factors at three levels. Under the optimal extraction condition (extraction time 180 min, ratio of water to solid 6, extraction temperature 100 °C, and extraction number 4), extraction yield of ASP was 5.6%. Rabbits were fed for 40 days with *A. sinensis* polysaccharides at a dose of 150 or 300 mg/kg body weight, respectively. At the end of 40 days, animals received cerebral ischemia reperfusion operation. CT perfusion imaging (CTP) analysis showed that rCBF and rCBV were significantly increased, whereas rMTT and rTTP were decreased in the ischemia cerebral tissue compared to CIR group rabbits. ASP significantly decreased oxidative damage, and increased antioxidant enzymes activities in brains of CIR animals. Moreover, ASP significantly enhanced the Ach, Na^+,K^+-ATPase, Ca^{2+},$Mg2^+$-ATPase and glucose levels, decreased AChE activity in brain tissue of the experimental animals. These results suggest a potent role of ASP in protection of brain oxidative injury in CIR animals.

1. Introduction

The roots of *Angelica sinensis* (Oliv.) Diels, also known in Chinese as Dang Gui or Dong Guai, a well-known herb belonging to the Umbelliferae family, has been used for more than 2000 years in China for various ailments. They have also been widely used as health foods for women's care in Asia (Tieraona, 2005), and were marketed in Europe and America as a dietary supplement (Deng et al., 2006).

The root of *A. sinensis*, also known as "Danggui", was a popular herbal medicine and widely used in China for gynecological diseases for a long time. Chemical and pharmacological studies of various extracts or compounds purified from the herbs were found to increase myocardial blood blow, reduce radiation damages and purify blood quality (Kim et al., 2002; Wang et al., 2001; Xie et al., 2001; Yim et al., 2000). *A. sinensis* was also demonstrated as mainly consisted of polysaccharides and had protective effect on gastrointestinal damage and hepatic injury (Ye, Koo, Li, Matsui, & Cho, 2001; Ye, Liu, Li, et al., 2001; Ye, Liu, Shin, et al., 2001; Ye, So, Liu, Shin, & Cho, 2003). In these options, different components in *A. sinensis* might involve different pharmacological activities.

Oxidative stress can be viewed as an imbalance between prooxidants and antioxidants, and both excess production of reactive oxygen species (ROS) and deficiency in cellular antioxidant defenses (e.g., glutathione; GSH) result in endogenous oxidative stress. Excessive ROS induce mitochondrial membrane pore transmission and impair the membrane integrity, leading to the release of cytochrome c, activation of caspase, and apoptosis (Ott, Gogvadze, Orrenius, & Zhivotovsky, 2007).

Cerebral ischemia/reperfusion insult produces cerebral damage via a complex cascade of pathophysiological events that evolve over a short period of time (Allen & Bayraktutan, 2009; Cheung, 2003). The brain is very susceptible to energy depriving injuries and the damage caused by oxidative stress, due to the high rate of oxidative metabolic activity, relatively low antioxidant capacity and inadequate neuronal cell repair activity (Chen, Du, & Zhang, 2000).

In the present study, we evaluate the inhibitory effect of *A. sinensis* polysaccharides against oxidative injury in cerebral ischemia reperfusion rabbits.

2. Materials and method

2.1. Extraction

The *A. sinensis* were cleaned and dried in a hot air oven at 60 °C and then cut into small slices. The sample was then boiled with

* Corresponding authors. Tel.: +86 021 23271699; fax: +86 021 63136856.
E-mail addresses: xftaohjtw@163.com (X. Tao), krdaifvt@163.com (K. Dai).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.02.007

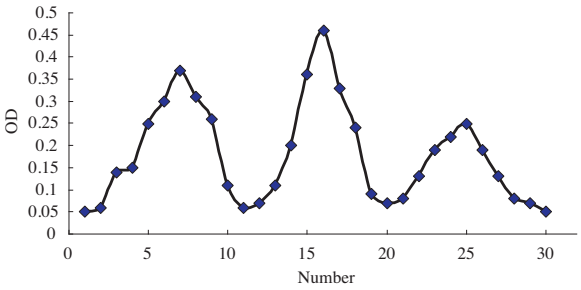

Fig. 1. Fraction of *A. sinensis* polysaccharides.

distilled water for some time and filtered with linen cloth. All crude extracts were centrifuged at 2000 × *g*, 4 °C for 15 min and evaporated in a rotary evaporator to reduce their volume. Then the crude extract was lyophilized and kept at 4 °C. The lyophilized samples were redissolved in water, and then the solutions were defatted and precipitated by acetone and 80% ethanol.

2.2. Fraction of A. sinensis polysaccharides

A. sinensis polysaccharides was dissolved in distilled water and centrifuged and loaded onto a cellulose DEAE-52 column (1.6 cm × 50 cm). Three fractions were obtained from the elutions, and they included the following: ASPF1, ASPF2 and ASPF3 (Fig. 1).

2.3. Animals and experimental design

Rabbits were provided by the Experimental Animal Center of Shanghai JiaoTong University. Rabbits were housed with free access to food and water under a natural day/night cycle. Rabbits were acclimated for 7 days before any experimental procedures. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Shanghai JiaoTong University. Rabbits were randomly assigned to the following four groups: sham, CIR and CIR + ASP groups. Animals in sham and CIR groups were fed with the basic diet for 40 days. Animals in CIR + ASP groups were fed with the basic diet and *A. sinensis* polysaccharides at a dose of 150 or 300 mg/kg body weight, respectively, for 40 days. After the last feeding, all animals received sham or cerebral ischemia reperfusion operation, respectively.

2.4. Animal model

The right common carotid arteries (CCA) was exposed through a midline neck incision after rabbits were anesthetized with chloral hydrate. The occipital artery branches of the external carotid artery (ECA) were then isolated, and these branches were dissected and coagulated. The internal carotid artery was isolated and carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was ligated. Next, a 4-cm length of 3-0 monofilament nylon suture was inserted via the proximal ECA into the internal carotid artery and thence into the circle of Willis, effectively occluding the MCA. After 45 min of occlusion, mono- filament was pulled out to resume the blood flow in the MCA and incision was closed. In the sham-operated group, the filament was only introduced into ECA but not advanced. Neurological deficits characterized by severe contralateral hemiparesis and ipsilateral Horner's syndrome were used as criteria for evaluating the ischemic insult.

2.5. CT perfusion imaging (CTP) protocol

CTP was performed on a GE LightSpeed VCT scanner (GE, Medical System, and Milwaukee, USA). Forty-five cine mode images were acquired 8 s after intravenous injection of 50 ml Iopromide (Ultravist, 370 mgI/ml, Schering company, Berlin, Germany) at a speed of 4 ml/s through a cubitus vein using a power injector. Technique parameters were as follows: 120 kV, 80 mA, 1 scan/s, matrix 512 × 512, field of view (FOV) 36 cm × 36 cm, thickness 5 mm × 8 i. Training of breath was performed before the examination to alleviate the influence of respiratory movement.

2.6. CTP data analysis

Images were handled with perfusion 3 software. CT perfusion parameters ((relative cerebral blood flow, rCBF), (cerebral blood volume, rCBV), (relative mean transit time, rMTT) and (relative mean transit time, rMTT)) were calculated by synthesis of cerebral perfusion maps.

2.7. Analysis of antioxidant enzymes activities

MDA, NO, GSH, SOD, CAT, GSH-Px, GR, Ach, AChE, Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}-ATPase and glucose levels were measured with commercially available kits. All analysis were performed according to the manufacturer's instructions.

2.8. Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was done using the SPSS11.0 software package. One way analysis of variance was used to establish whether the difference among the three groups was statistically significant. *P* value less than 0.05 was considered statistically significant.

3. Results

In Fig. 2, (1) the extraction yield of the polysaccharides increased with increasing extraction time from 30 min to 180 min. When extraction time was 180 min, the extraction yield of the polysaccharides was the highest.

In Fig. 2, (2) the extraction yield of the polysaccharides increased with increasing ratio of water to solid from 2 to 6. When ratio of water to solid was 6, the extraction yield of the polysaccharides was the highest.

In Fig. 2, (3) the extraction yield of the polysaccharides increased with increasing extraction temperature from 40 to 90 °C. When extraction temperature was 90 °C, the extraction yield of the polysaccharides was the highest.

In Fig. 2, (4) the extraction yield of the polysaccharides increased with increasing extraction number from 1 to 5. When extraction number was 5, the extraction yield of the polysaccharides was the highest.

According to the orthogonal method (Ding, Noritomi, & Nagahama, 2001; Zhang et al., 2011), effect of the extraction parameters on the yield of polysaccharides were calculated. It can be clearly seen the result were as follows: to obtain a high extraction yield, the optimum composition are extraction time 180 min, ratio of water to solid 6, extraction temperature 100 °C, and extraction number 4.

The rCBF, rCBV, rMTT and rTTP in the ischemia cerebral tissue of sham, CIR and CIR + ASP rabbits were described in Fig. 3. In CIR rabbits, rCBF and rCBV were significantly decreased, whereas rMTT and rTTP were increased in the ischemia cerebral tissue compared to sham rabbits ($p < 0.05$). Treatment of CIR rabbits with *A. sinensis* polysaccharides significantly increased rCBF and rCBV, and decreased the rMTT and rTTP in the ischemia cerebral tissue of CIR + ASP groups rabbits compared to CIR group rabbits ($p < 0.01$).

The effects of *A. sinensis* polysaccharides at two dose levels (150 and 300 mg/kg, p.o.) on ischemia cerebral tissue MDA and

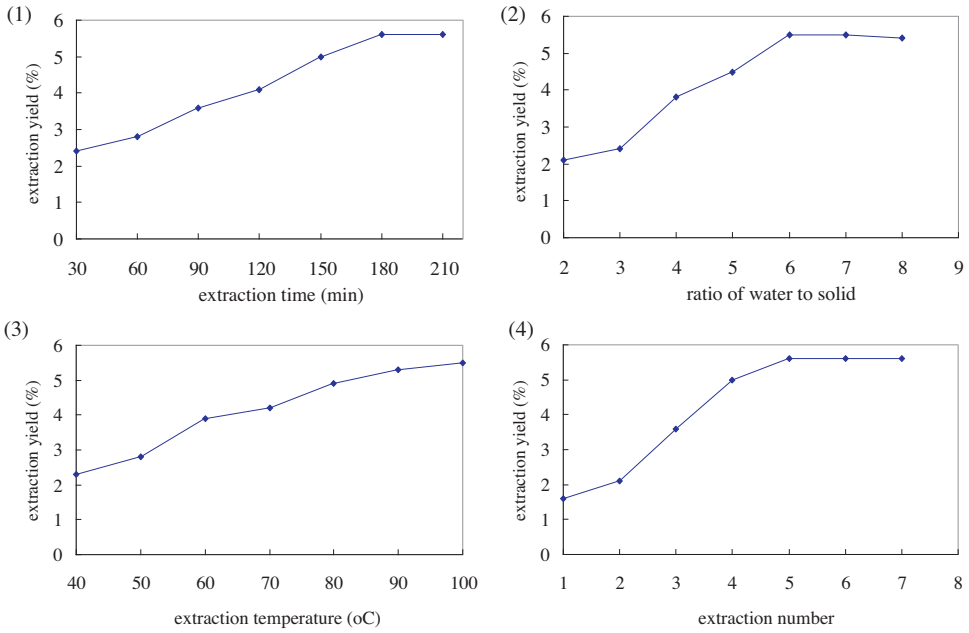

Fig. 2. Effect of extraction parameters (extraction time (1), ratio of water to solid (2), extraction temperature (3), extraction number (4)) on extraction yield of *A. sinensis* polysaccharides.

NO levels in CIR rabbits are shown in Fig. 4. CIR caused significant rise in ischemia cerebral tissue MDA and NO levels of CIR group compared to sham group. Administration of *A. sinensis* polysaccharides at two dose levels (150 and 300 mg/kg, p.o.) attenuated the decreased levels of the ischemia cerebral tissue MDA and NO, and caused a subsequent recovery toward normalization (Fig. 4).

The effects of *A. sinensis* polysaccharides at two dose levels (150 and 300 mg/kg, p.o.) on ischemia cerebral tissue GSH level in CIR rabbits are shown in Fig. 4. CIR caused significant decrease in ischemia cerebral tissue GSH level of CIR group compared to sham group. Administration of *A. sinensis* polysaccharides at two dose levels (150 and 300 mg/kg, p.o.) attenuated the increased levels of the ischemia cerebral tissue GSH and caused a subsequent recovery toward normalization (Fig. 4).

The effects of *A. sinensis* polysaccharides at two dose levels (150 and 300 mg/kg, p.o.) on ischemia cerebral tissue SOD, CAT, GSH-Px and GR activities in CIR rabbits are shown in Fig. 5. CIR caused significant decrease in ischemia cerebral tissue SOD, CAT, GSH-Px and GR activities of CIR group. Administration of *A. sinensis* polysaccharides at two dose levels (150 and 300 mg/kg, p.o.) attenuated the increased levels of the ischemia cerebral tissue SOD, CAT, GSH-Px and GR activities and caused a subsequent recovery toward normalization (Fig. 5).

Fig. 6 illustrates the Ach, AChE, Na^+,K^+-ATPase, Ca^{2+},$Mg2^+$-ATPase and glucose levels in the experimental animals. The Ach, Na^+,K^+-ATPase, Ca^{2+},$Mg2^+$-ATPase and glucose levels in CIR groups were significantly lower than those in sham group, whereas AChE

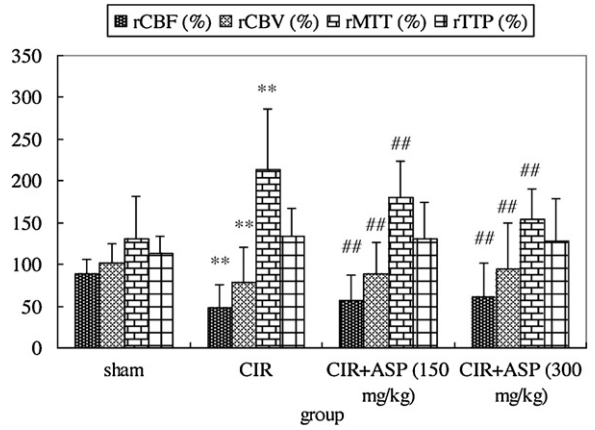

Fig. 3. Effect of *A. sinensis* polysaccharides on rCBF, rCBV, rMTT and rTTP $^{**}P < 0.01$, compared with sham group; $^{\#\#}P < 0.01$, compared with CIR group.

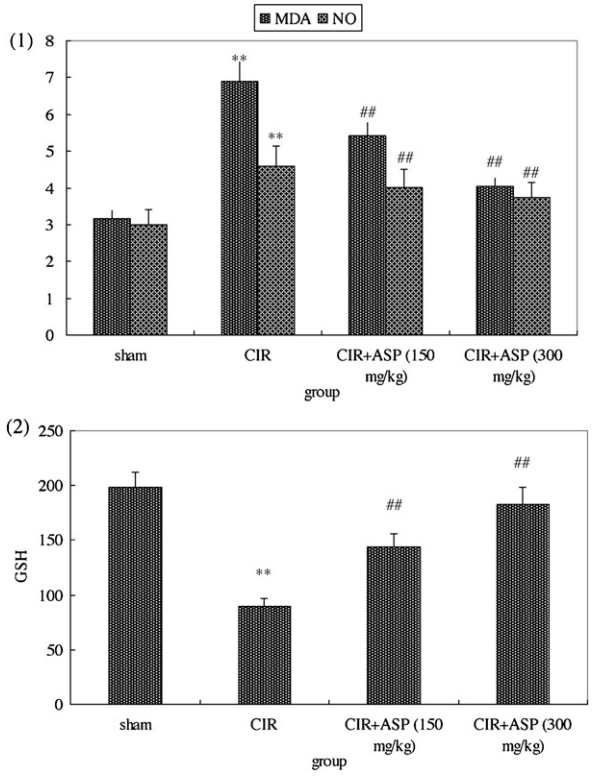

Fig. 4. Effect of *A. sinensis* polysaccharides on MDA, NO and GSH levels $^{**}P < 0.01$, compared with sham group; $^{\#\#}P < 0.01$, compared with CIR group.

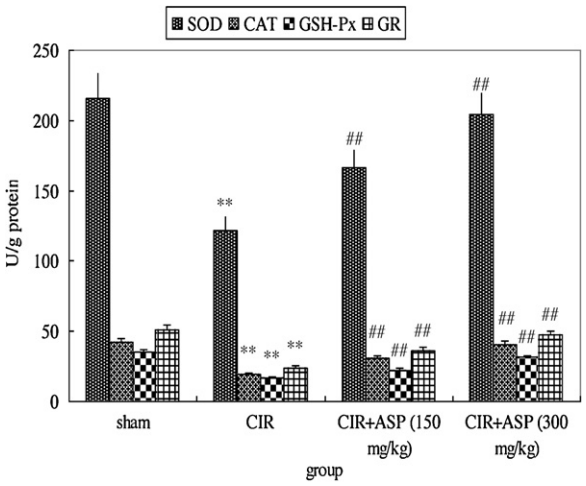

Fig. 5. Effect of *A. sinensis* polysaccharides on SOD, CAT, GSH-Px and GR activities $^{**}P < 0.01$, compared with sham group; $^{\#\#}P < 0.01$, compared with CIR group.

in CIR group was markedly higher. These effects were dramatically reversed by treatment with *A. sinensis* polysaccharides.

4. Discussion

Computed tomography (CT) is generally performed before starting the therapy in order to exclude the presence of bleeding and tumors. With cerebral perfusion imaging, it is possible to diagnose ischemia early, as well as gather information about the extension and severity of the ischemia. Perfusion and diffusion-weighted MR examinations are more sensitive imaging methods in the diagnosis of acute ischemia as compared to routine brain CT. Ischemic areas can be determined within minutes or hours. Both imaging methods can provide rather useful information for determining the tissue under risk that is progressing to infarct, particularly when used together (Eastwood, Lev, & Provenzale, 2003). In this study, we investigated the effect of *A. sinensis* polysaccharide on CIR-induced brain oxidative injury as well as its related mechanism. The results showed that *A. sinensis* polysaccharide could enhance rCBF (%) and rCBV (%), and decrease rMTT (%) levels in CIR rats. Interestingly, no variation in rTTP (%) levels was observed in CIR rats.

In the pathophysiology of cerebral ischemia/reperfusion, oxidative stress is one of the mechanisms that exacerbates brain injury (Allen & Bayraktutan, 2009; Doyle, Simon, & Stenzel-Poore, 2008). Ischemia/reperfusion insult induces damage in a consecutive pattern by immediate and direct production of reactive oxygen, including hydroxyl radical, superoxide anion, and nitrogen free radical, NO (Warner, Sheng, & Batinić-Haberle, 2004). Brain reperfusion after ischemia frequently results in neuronal death, which occurs preferentially in some brain regions. This neuronal degeneration has been associated with ROS, which react with cellular macromolecules such as lipids, proteins and nucleic acids leading to oxidative damage of the neurons (Halliwell, 1992; Negishi, Ikeda, Nara, & Yamori, 2001). Thus the endogenous antioxidant enzyme activity of the brain impaired by I/R is particularly important and measurement of those antioxidant enzymes after reperfusion can assess the vulnerability of the particular areas of the brain (Macdonald & Stoodley, 1998). Novel therapeutic neuroprotective strategies support the applications of ROS scavengers and induction of endogenous antioxidants drugs, such as natural antioxidants, e.g. plant derived polyphenolic compounds, in mono therapy, or as part of an antioxidant cocktail formulation, for the treatment of neurodegenerative diseases (Gali, Shukitt-Hale, Youdim, & Joseph, 2002; Homi, Freitas, Curi, Velasco, & Junior, 2002; Ishige, Schubert, & Sagara, 2001; Mandel & Yodium, 2004; Slikker et al., 1999).

ROS triggered oxidative stress shown by the increase in MDA, the product of lipid peroxidation. As a decomposition product, MDA has been used as a biomarker of lipid peroxidation for several decades, and furthermore, the increase of MDA has been considered as a key feature in ALD (Cherubini, Ruggiero, & Polidori, 2005; Lykkesfeldt, 2007). NO is a gaseous, ubiquitous neurotransmitter with multiple functions in the brain and peripheral nervous system and an important mediator of vascular homeostasis and blood flow. It can improve neuronal survival, inhibit platelet aggregation and neutrophil adhesion, and scavenge reactive free

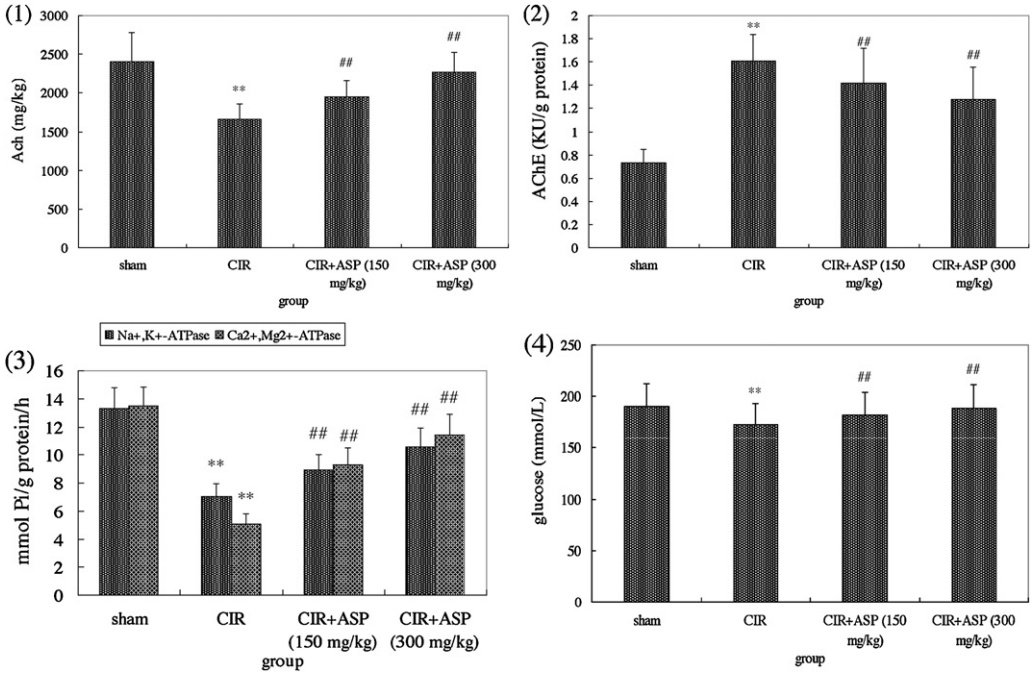

Fig. 6. Effect of *A. sinensis* polysaccharides on Ach, AChE, Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}-ATPase and glucose levels $^{**}P < 0.01$, compared with sham group; $^{\#\#}P < 0.01$, compared with CIR group.

radicals, thus reducing the ischemic injury (Mohanakumar et al., 2002; Stagliano, Dietrich, Prado, Green, & Busto, 1997). However, a concomitant surge in production of superoxide and NO after reperfusion may lead to formation of peroxynitrite, a powerful oxidant (Beckman, Ye, Chen, & Conger, 1996). So far, evidences have indicated that NO may be linked both to protective and toxic effects after ischemia/reperfusion (Jung et al., 2006). GSH, the most abundant thiol in mammals, was discovered a century ago, and its central function in detoxification and protection against oxidants was recognized 50 years ago (Hwang, Sinskey, & Lodish, 1992). Apart from GSH, a great abundance of antioxidant enzymes in livers including SOD, CAT, GSH-Px and GR can also weaken oxidative stress (Sun, 1990). The increased activities of SOD, CAT, GPx, GR, and GST are known to serve as protective responses to eliminate reactive free radicals (Cheung, Zheng, Li, Richardson, & Lam, 2001). Comparing with CIR groups, it could be noticed that the rats' brain MDA and NO concentration stayed low for a longer time in *A. sinensis* polysaccharide treatment groups, however, GSH level was higher, implicating that ASP could decrease oxidative injury in CIR rats. In addition, *A. sinensis* polysaccharide treatment had significantly enhanced cerebral SOD, CAT, GSH-Px and GR activities in CIR rats. Therefore, the potent radical scavenging properties or LPO inhibiting ability of *A. sinensis* polysaccharide protecting the neurons from oxidative stress may provide useful therapeutic agent for the treatment of neurodegenerative diseases such as I/R induced oxidative stress.

ACh was endogenous neurotransmitters of cholinergic nerve conduction. Its level in brain was closely associated with brain function. To examine Ach level may reflect the function of cholinergic nerve in brain. In this study, *A. sinensis* polysaccharide increased Ach level in brain of CIR+ASP groups, suggesting that *A. sinensis* polysaccharide inhibited ACh decomposition by decreasing AChE activity. Ca^{2+}/Mg^{2+}-ATPase and Na^{+}/K^{+}-ATPase catalyze the hydrolysis of ATP that is coupled to the active transport of Ca^{2+}/Mg^{2+} and Na^{+}/K^{+} across the cell membrane (Aubier & Viires, 1998; Goto, Miura, & Iijima, 1996; Mohandas & Shohet, 1978; Skou, 1965). Collectively, ion pumps use a large percentage of the total cellular energy, e.g., Na^{+}/K^{+}-ATPase is responsible for 5–40% of total ATP turnover depending on cell type (Clausen, 1986). Maintenance of the cation gradient by these ATPase enzymes is of fundamental importance in the control of hydration, volume, nutrient uptake and fluidity of cells. Na^{+},K^{+}-ATPase is a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It is present at high concentrations in brain cellular membranes, consuming about 40–50% of the ATP generated in this tissue (Erecinska & Silver, 1994). It has been demonstrated that this enzyme is susceptible to free radical attack (Lees, 1993). Besides, there are some reports showing that Na^{+},K^{+}-ATPase activity is decreased in cerebral ischemia (Wyse et al., 2000), in epilepsy (Grisar, Guillaume, & Delgado-Escueta, 1992) and in various chronic neurodegenerative disorders (Lees, 1993). In this study, *A. sinensis* polysaccharide may enhance Na^{+},K^{+}-ATPase, Ca^{2+},Mg^{2+}-ATPase activities and glucose level, indicating that *A. sinensis* polysaccharide may enhance glucose metabolism in brain.

In conclusion, *A. sinensis* polysaccharide has been divided into three fractions. The purity of the *A. sinensis* leached according to the procedure above was calculated as 96.7%. *A. sinensis* polysaccharide protect IR-induced brain oxidative injury by its enhancing antioxidant activity, regulating metabolism and decreasing AChE activity.

References

Allen, C. L., & Bayraktutan, U. (2009). Oxidative stress and its role in the pathogenesis of ischaemic stroke. *International Journal of Stroke*, *4*, 461–470.

Aubier, M., & Viires, N. (1998). Calcium ATPase and respiratory muscle functions. *European Respiratory Journal*, *11*, 758–766.

Beckman, J. S., Ye, Y. Z., Chen, J., & Conger, K. A. (1996). The interactions of nitric oxide with oxygen radicals and scavengers in cerebral ischemic injury. *Advances in Neurology*, *71*, 339–350.

Chen, Y. H., Du, G. H., & Zhang, J. T. (2000). Salvianolic acid B protects brain against injuries caused by ischemia–reperfusion in rats. *Acta Pharmacologica Sinica*, *21*, 463–466.

Cherubini, A., Ruggiero, C., & Polidori, M. C. (2005). Potential markers of oxidative stress in stroke. *Free Radical Biology & Medicine*, *39*, 841–852.

Cheung, C. C., Zheng, G. J., Li, A. M. Y., Richardson, B. J., & Lam, P. K. S. (2001). Relationship between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels *Perna viridis*. *Aquatic Toxicology*, *52*, 189–203.

Cheung, R. T. (2003). The utility of melatonin in reducing cerebral damage resulting from ischemia and reperfusion. *Journal of Pineal Research.*, *34*, 153–160.

Clausen, T. (1986). Regulation of active Na^{+}, K^{+} transport in muscle. *Physiological Reviews*, *66*, 542–576.

Deng, S., Chen, S. N., Yao, P., Nikolic, D., van Breemen, R. B., Bolton, J. L., et al. (2006). Serotonergic activity-guided phytochemical investigation of the roots of *Angelica sinensis*. *Journal of Natural Products*, *69*, 536–541.

Ding, F. P., Noritomi, H., & Nagahama, K. (2001). Optimization of fermentation conditions for preparation of polygalacturonic acid transeliminase by Erwinia carotovora IFO3830. *Biotechnology Progress*, *17*, 311–317.

Doyle, K. P., Simon, R. P., & Stenzel-Poore, M. P. (2008). Mechanisms of ischemic brain damage. *Neuropharmacology.*, *55*, 310–318.

Eastwood, J. D., Lev, M. H., & Provenzale, J. M. (2003). Perfusion CT with iodinated contrast material. *American Journal of Roentgenology*, *180*, 3–12.

Erecinska, M., & Silver, I. A. (1994). Ions and energy in mammalian brain. *Progress in Neurobiology*, *43*, 37–71.

Gali, R. L., Shukitt-Hale, B., Youdim, K. A., & Joseph, J. A. (2002). Fruit polyphenolics and brain aging: Nutritional interventions targeting age-related neuronal and behavioral deficits. *Annals of the New York Academy of Sciences*, *959*, 128–132.

Goto, Y., Miura, M., & Iijima, T. (1996). Extrusion mechanisms of intracellular Ca^{2+} in human aortic endothelial cells. *European Journal of Pharmacology*, *314*, 185–192.

Grisar, T., Guillaume, D., & Delgado-Escueta, A. V. (1992). Contribution of Na^{+}, K^{+}-ATPase to focal epilepsy: A brief review. *Epilepsy Research*, *12*, 141–149.

Halliwell, B. (1992). Reactive oxygen species and the central nervous system. *Journal of Neurochemistry*, *59*, 1609–1623.

Homi, H. M., Freitas, J. J. S., Curi, R., Velasco, I. T., & Junior, B. A. S. (2002). Changes in superoxide dismutase and catalase activities of rat brain regions during early global transient ischemia/reperfuion. *Neuroscience Letters*, *333*, 37–40.

Hwang, C., Sinskey, J., & Lodish, H. F. (1992). Oxidized redox state of glutathione in the endoplasmic-reticulum. *Science*, *257*, 1496–1502.

Ishige, K., Schubert, D., & Sagara, Y. (2001). Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radical Biology & Medicine*, *30*, 433–446.

Jung, K. H., Chu, K., Ko, S. Y., Lee, S. T., Sinn, D. I., Park, D. K., et al. (2006). Early intravenous infusion of sodium nitrite protects brain against in vivo ischemia–reperfusion injury. *Stroke*, *37*, 2744–2750.

Kim, S. H., Lee, S. E., Oh, H., Kim, S. R., Yee, S. T., Yu, Y. B., et al. (2002). The radioprotective effects of buzhong-yi-qi-tang: A prescription of traditional Chinese medicine. *American Journal of Chinese Medicines*, *30*(1), 127–137.

Lees, G. J. (1993). Contributory mechanisms in the causation of neurodegenerative disorders. *Neuroscience*, *54*, 287–322.

Lykkesfeldt, J. (2007). Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. *Clinica Chimica Acta*, *380*, 50–58.

Macdonald, R. L., & Stoodley, M. (1998). Pathophysiology of cerebral ischemia. *Neurologia Medico-Chirurgica*, *38*, 1–11.

Mandel, S., & Yodium, M. B. H. (2004). Catechin polyphenol: Neurodegeneration and neuroprotection in neurodegenerative diseases. *Free Radical Biology & Medicine.*, *37*, 304–317.

Mohanakumar, K. P., Thomas, B., Sharma, S. M., Muralikrishnan, D., Chowdhury, R., & Chiueh, C. C. (2002). Nitric oxide: An antioxidant and neuroprotector. *Annals of the New York Academy of Sciences*, *962*, 389–401.

Mohandas, N., & Shohet, S. B. (1978). Control of red cell deformability and shape. *Current Topics in Hematology*, *1*, 71–125.

Negishi, H., Ikeda, K., Nara, Y., & Yamori, Y. (2001). Increased hydroxyl radical in the hippocampus of stroke prone spontaneously hypertensive rats during transient ischemia and recirculation. *Neuroscience Letters*, *306*, 206–208.

Ott, M., Gogvadze, V., Orrenius, S., & Zhivotovsky, B. (2007). Mitochondria, oxidative stress and cell death. *Apoptosis*, *12*, 913–922.

Skou, J. C. (1965). Enzymatic basis for active transport of Na^{+} and K^{+} across cell membrane. *Physiological Reviews*, *45*, 596–617.

Slikker, W., Youdim, M. B., Palmer, G. C., Hall, E., Williams, C., & Trembly, B. (1999). The future of neuroprotection. *Annals of the New York Academy of Sciences*, *890*, 529–533.

Stagliano, N. E., Dietrich, W. D., Prado, R., Green, E. J., & Busto, R. (1997). The role of nitric oxide in the pathophysiology of thromboembolic stroke in the rat. *Brain Research*, *759*, 32–40.

Sun, Y. (1990). Free-radicals, antioxidant enzymes, and carcinogenesis. *Free Radical Biology & Medicine*, *8*, 583–599.

Tieraona, L. D. (2005). Menopause: A review of botanical dietary supplements. *The American Journal of Medicine*, *118*, 98S–108S.

Wang, X., Wei, L., Ouyang, J. P., Muller, S., Gentils, M., Cauchois, G., et al. (2001). Effects of an angelica extract on human erythrocyte aggregation, deformation and osmotic fragility. *Clinical Hemorheology and Microcirculation*, *24*(3), 201–205.

Warner, D. S., Sheng, H., & Batinić-Haberle, I. (2004). Oxidants, antioxidants and the ischemic brain. *The Journal of Experimental Biology*, *207*, 3221–3231.

Wyse, A. T. S., Streck, E. L., Worm, P., Wajner, A., Ritter, F., & Netto, C. A. (2000). Preconditioning prevents the inhibition of Na^+, K^+-ATPase activity after brain ischemia. *Neurochemical Research*, *25*, 971–975.

Xie, F., Li, X., Sun, K., Chu, Y., Cao, H., Chen, N., et al. (2001). An experimental study on drugs for improving blood circulation and removing blood stasis in treating mild chronic hepatic damage. *Journal of Traditional Chinese Medicines*, *21*(3), 225–231.

Ye, Y. N., Koo, M. W., Li, Y., Matsui, H., & Cho, C. H. (2001). *Angelica sinensis* modulates migration and proliferation of gastric epithelial cells. *Life Sciences*, *68*(8), 961–968.

Ye, Y. N., Liu, E. S., Li, Y., So, H. L., Cho, C. C., Sheng, H. P., et al. (2001). Protective effect of polysaccharides enriched fraction from *Angelica sinensis* on hepatic injury. *Life Sciences*, *69*(6), 637–646.

Ye, Y. N., Liu, E. S., Shin, V. Y., Koo, M. W., Li, Y., Wei, E. Q., et al. (2001). A mechanistic study of proliferation induced by *Angelica sinensis* in a normal gastric epithelial cell line. *Biochemical Pharmacology*, *61*(11), 1439–1448.

Ye, Y. N., So, H. L., Liu, E. S., Shin, V. Y., & Cho, C. H. (2003). Effect of polysaccharides from *Angelica sinensis* on gastric ulcer healing. *Life Sciences*, *72*(8), 925–932.

Yim, T. K., Wu, W. K., Pak, W. F., Mak, D. H., Liang, S. M., & Ko, K. M. (2000). Myocardial protection against ischaemia-reperfusion injury by a *Polygonum multiflorum* extract supplemented 'Dang-Gui decoction for enriching blood', a compound formulation, ex vivo. *Phytotherapy Research*, *14*(3), 195–199.

Zhang, Y., Wang, L. Z., Zhang, A. Q., Song, Y. H., Li, X. F., Wu, X. B., et al. (2011). Impact of electrolyte additives (alkali metal salts) on the capacitive behavior of NiO-based capacitors. *Korean Journal of Chemical Engineering*, *28*, 608–612.