Antioxidative effects of *Panax notoginseng* saponins in brain cells

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**Abstract**

Oxidative stress resulting from accumulation of reactive oxygen species (ROS) is involved in cell death associated with neurological disorders such as stroke, Alzheimer's disease and traumatic brain injury. Antioxidant compounds that improve endogenous antioxidant defenses have been proposed for neural protection. The purpose of this study was to investigate the potential protective effects of total saponin in leaves of *Panax notoginseng* (LPNS) on oxidative stress and cell death in brain cells in vitro. Lactate dehydrogenase (LDH) assay indicated that LPNS (5 μg/ml) reduced H\(_2\)O\(_2\)-induced cell death in primary rat cortical astrocytes (23 ± 8% reduction in LDH release vs. control). Similar protection was found in oxygen and glucose deprivation/reoxygenation induced SH-SYSY (a human neuroblastoma cell line) cell damage (78 ± 7% reduction vs. control). The protective effects of LPNS in astrocytes were associated with attenuation of reactive oxygen species (ROS) accumulation. These effects involved activation of Nrf2 (nuclear translocation) and upregulation of downstream antioxidant systems including heme oxygenase-1 (HO-1) and glutathione S-transferase pi 1 (GSTP1). These results demonstrate for the first time that LPNS has antioxidant effects which may be neuroprotective in neurological disorders.

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**Introduction**

Oxidative stress is an imbalance between formation of reactive oxygen and nitrogen species and endogenous antioxidant defenses and repair capacity (Crack and Taylor, 2005). It results from accumulation of reactive oxygen species (ROS) and is involved in cell death observed in multiple pathological processes including stroke, brain tumor and neurodegenerative diseases (Lotharius et al., 2005; Resende et al., 2008). Reactive oxygen and nitrogen species change cellular responses through diverse mechanisms. At low levels, they are signaling molecules, while at high levels they damage organelles, particularly the mitochondria. Oxidative damage and associated mitochondrial dysfunction may result in energy depletion, accumulation of cytotoxic mediators and cell death.

Various tissues differ in susceptibility to oxidative stress. Thus, the brain is very susceptible to energy deprivation and damage induced by oxidative stress, due to a high rate of oxidative metabolic activity and inadequate neuronal cell repair activity (Braughler and Hall, 1989). Astrocytes maintain high intracellular concentrations of certain antioxidants, such as vitamins E and C and antioxidant enzymes, which play a major neuroprotective role in brain resistance to the deleterious effects of superoxides (Lotharius et al., 2005; Papadopoulos et al., 1998; Wilson, 1997). In reactive gliosis, the neuroprotective role of astrocytes may be accentuated because of increases in a number of activities: expression of antioxidant enzymes; transport and metabolism of glucose that yields reducing equivalents for antioxidant regeneration and lactate for neuronal metabolism; synthesis of glutathione; and recycling of vitamin C. Although astrocytes are generally less susceptible to oxidative injury than neurons, there is strong evidence that oxidative stress also alters astrocyte function (Peuchon et al., 1997). Astrocytic apoptosis is observed in brain injuries caused by trauma and ischemia. In particular, they are extremely vulnerable to hydrogen peroxide (H\(_2\)O\(_2\)) induced damage (Giffard and Swanson, 2005; Takuma et al., 2004). Therefore, protection of astrocytes from oxidative stress appears essential to maintain cerebral antioxidant competence and to prevent neuronal damage as well as to facilitate neuronal recovery (Hamdi et al., 2011).

Compounds which act to improve endogenous antioxidant defenses, have been proposed to serve as an avenue for therapeutic attempts aimed at limiting neural degeneration and improving neurological recovery.

In recent years, researchers have found that many herbal drugs and their extracts have antioxidative effects on brain injury. *Panax*
noginseng (Burk.) F.H. Chen (Sanqi), a traditional Chinese materia medica, is widely used in Chinese medicine to arrest internal and external hemorrhages, eliminate blood stasis, improve blood circulation, disperse bruises, reduce swelling and pain (Lei and Chiou, 1986). Ginsenosides or dammarane-type triterpenoidal saponins, the main bioactive constituents of P. notoginseng, are effective in preventing and treating cerebro- and cardiovascular diseases. Clinically, P. notoginseng Saponin (PNS) is used in the treatment of acute ischemic stroke (Chen et al., 2008; He et al., 2011) and cardiovascular disease (Chan et al., 2002; Chen et al., 2011). These compounds are also immunoregulatory, possessing properties such as hepatoprotection and anti-carcinogenesis (Ng, 2006). In experimental studies, a wide range of pharmacological activities of PNS had been reported include anti-inflammatory, hemostatic, hypolipidemic, hepatoprotective, renoprotective, and estrogen-like activities (Lei and Chiou, 1986; Ng, 2006). Recent research found that panaxatriol saponins protect against focal cerebral ischemia in rat by reducing cerebral edema, up-regulating heat shock protein HSP70 expression and down-regulating transferrin (Yao and Li, 2002). Antioxidative effects of the water extract of P. notoginseng have also been reported in PC12 cells (Choi et al., 2010).

Although P. notoginseng has been shown to protect against neurological diseases, the underlying mechanisms remain poorly understood, especially with regards to any antioxidant effect in astrocytes. Furthermore, while the therapeutic effects of P. notoginseng has been focused on its saponin of root, the pharmacological effects of other parts such as saponin in leaves of P. notoginseng (PNS), rich in protopanaxadiol saponins, are largely unknown. It is of importance to explore the effect and mechanism of PNS as a novel therapeutics for neurological disorders related to oxidative stress. This present study examines the antioxidative effects of PNS in primary astrocytes and a neuroblastoma cell line, SH-SYSY, and examines the effect of PNS on the transcription factor Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a master regulator of antioxidative defense mechanisms, and downstream enzyme.

**Materials and methods**

Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT). Dulbecco's modified Eagle's medium (DMEM), L-glutamine, antibiotic-Antimycotic (100×), trypsin-EDTA, H₂O₂ and collagen were obtained from Invitrogen (Carlsbad, CA, USA).

The total saponin from the leaves of Panax notoginseng was a gift from Yunnan Hongyun Bioengineering Technology Co., Ltd. (Kunming, China). The fingerprint analysis of the PNS sample was performed as described by Wagner et al. (2011) with slight modification. Five standard substances (ginsenoside Rb₁, Rb₂, Rc, Re and Rd) were purchased from the National Institute for the Pharmaceutical and Biological Products (Beijing, PR China). The tested sample was dissolved in methanol and the solution was filtered using a 0.45 μm Millipore® filtration unit. The HPLC system (SHIMADZU LC-2010A, SHIMADZU, Japan) was equipped with a binary solvent delivery system and an auto-ssa. 20 μl of the sample were separated on an Agilent Extend-C18 column (4.6 mm × 250 mm, 5 μm; Agilent Technologies, USA) maintained at 60°C. The mobile phase consisted of H₂O (A) and CH₃CN (B) went through a gradient as 15–30% (v/v) B for 20 min, 30–50% B for 20 min, and 35–50% B for 15 min. The flow rate was fixed at 1.0 ml/min and the DAD detector was set at 203 nm.

The PNS-fingerprints (Fig. 1) were characterized by two dominating peaks of ginsenoside Re (peak 2, Rt = 29.18) and ginsenoside Rb₃ (peak 4, Rt = 32.06), with much lower contents of ginsenoside Rb₁ (peak 1, Rt = 27.33), ginsenoside Rb₂ (peak 3, Rt = 31.19), and ginsenoside Rd (peak 5, Rt = 36.04) according to standard substance fingerprint. The other peak at Rt = 47.0 may be assigned to panaxadiol-3 or -triol. The content of these 5 compounds in PNS was calculated as the peak area of the compound relative to the total peak area, respectively (Table 1).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min.)</th>
<th>Compound</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.33</td>
<td>Ginsenoside Rb₁</td>
<td>2.80</td>
</tr>
<tr>
<td>2</td>
<td>29.18</td>
<td>Ginsenoside Rc</td>
<td>11.72</td>
</tr>
<tr>
<td>3</td>
<td>31.19</td>
<td>Ginsenoside Rb₂</td>
<td>2.61</td>
</tr>
<tr>
<td>4</td>
<td>32.06</td>
<td>Ginsenoside Rb₃</td>
<td>17.38</td>
</tr>
<tr>
<td>5</td>
<td>36.04</td>
<td>Ginsenoside Rd</td>
<td>3.87</td>
</tr>
</tbody>
</table>

The contents of different compounds in PNS as determined by HPLC (see Fig. 1). 

**Fig. 1.** HPLC-fingerprint analysis of LPNS. 1, ginsenoside Rb₁; 2, ginsenoside Rc; 3, ginsenoside Rb₂; 4, ginsenoside Rb₃ and 5, ginsenoside Rd.
Cell cultures

Astrocytes were isolated and cultured from the cortex of 1–2 day old Sprague-Dawley rats using a modified version of the procedures previously described (McCarthy and de Vellis, 1980). This study followed the Animal Use and Care Guidelines issued by the National Institutes of Health using a protocol approved by the Animal Use and Care Committee at the University of Michigan. Cells were collected and seeded on collagen-coated 48-well-plate or 10 cm dishes. The culture medium was changed every 48 h in Dulbecco’s Modified Eagle’s Medium (DMEM) with 4.5 g/l glucose and L-glutamine containing 10% FBS, 2% glucose and 1% antibiotic-antimycotic agents. Cells were grown in a humidified incubator with 95% air-5% CO₂ at 37°C. SH-SY5Y cells (ATCC; Manassas, VA, USA) were cultured as previously described (Sabirzhanova et al., 2009). Briefly, cells were grown in DMEM supplemented with 1% FBS in a humidified incubator with 95% air-5% CO₂ at 37°C. The cells were passaged (1:12–16) twice a week.

H₂O₂ treatment

A 30% solution of H₂O₂ was diluted in FBS-free DMEM and added to the cultures to a final concentration of 0.5 mM. Prior to H₂O₂ insult, cell cultures were pretreated with vehicle (DMSO) or LPNS at 2.5, 5 or 10 µg/ml. The medium was then replaced and cells incubated with FBS-free DMEM with or without freshly made 0.5 mM H₂O₂ for 1h or 4h. In each experiment, cultures exposed to H₂O₂ were compared with normal controls (FBS-free DMEM not containing H₂O₂) and vehicle-treated cells exposed to H₂O₂.

Oxygen-glucose deprivation/reoxygenation

Oxygen-glucose deprivation (OGD) was performed as previously described (Goldberg and Choi, 1993; Shen et al., 2008). Briefly, SH-SY5Y cells were pretreated with vehicle or LPNS at 2.5, 5 or 10 µg/ml in culture medium for 24 h, then washed twice and incubated in glucose-free DMEM (Life Technologies, Grand Island, New York, USA). The cultures were transferred into an anaerobic chamber filled with a gas mixture of 95% N₂/5% CO₂ at 37°C for 5 h. At the end of the OGD, cells were incubated in regular culture medium and returned to normoxic conditions (37°C in a humidified 95% air-5% CO₂ atmosphere) for another 24 h. In each experiment, cultures exposed to OGD were compared with normoxic controls (supplied with DMEM containing glucose and maintained in standard incubation conditions) and vehicle-treated cells exposed to OGD.

Lactate dehydrogenase release

Cell injury was assessed by measuring extracellular lactate dehydrogenase (LDH) release. Primary astrocyte and SH-SY5Y cells were grown to confluence on polycarbonate 48-well plates. In the first set of experiments, cells were then pretreated with vehicle, 2.5, 5 or 10 µg/ml LPNS for 24 h in culture medium before exposure to 0.5 mM H₂O₂ (4 h) or OGD. LDH release into the medium was measured using CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI, USA), at 492 nm with a microplate reader (ELX, Bio-Tek, Winooski, VT, USA). The amount of LDH released from each test sample was expressed as a percentage of the control and vehicle-treated cells exposed to H₂O₂. In a second set of experiments, astrocytes were treated with 5 µg/ml LPNS for 1–48 h before exposure to 0.5 mM H₂O₂ to examine the time course of protection.

Intracellular ROS production

Intracellular ROS production was detected using an OxiSelect™ Intracellular ROS Assay Kit (Cell Biolabs, San Diego, CA, USA). The assay employs a cell-permeable fluorogenic probe 2',7'-dichlorodihydrofluorescin diacetate (DCHF-DA) which is deacetylated by intracellular esterases and then oxidized by ROS to a fluorescent compound 2',7'-dichlorofluorescin (DCF). Astrocytes were treated with LPNS at 2.5, 5, or 10 µg/ml for 24 h and then gently washed 3x with Dulbecco’s PBS. Cells were then incubated at 37°C for 30 min after adding 100 µl of DCFH-DA media solution followed by exposure to 0.5 mM H₂O₂ in FBS-free DMEM for 1 h. Cells were then washed 3x with Dulbecco’s PBS before adding 200 µl of cell lysis buffer. Five minutes later, 150 µl of the cell lysate was transferred to a 96-well plate and read with a fluorometric plate reader at 480 nm/530 nm.

Western blotting studies

Astrocytes pretreated with 5 µg/ml LPNS for 24 h were collected and lysed with RIPA buffer (Sigma, Saint Louis, MO, USA) for heme oxygenase (HO-1) detection. Supernatants were collected and protein concentration determined by BCA assay kit (Thermo Fisher Scientific, Rockford, IL, USA). Cells treated with 5 µg/ml LPNS and harvested at 1 h were used to examine nuclear translocation of Nrf2. Nuclear fractions were isolated using a cell fractionation kit (Calbiochem, La Jolla, CA, USA).

Fifty µg of each protein sample was run through a 10% SDS-PAGE gel and transferred onto a nitrocellulose membrane (Amersham, Piscataway, NJ, USA). After being washed 3x with PBS buffer, membranes were blocked in 5% non-fat dry milk for 1 h and incubated with rabbit anti-rat HO-1 (Assay Design, Ann Arbor, MI, USA) or rabbit anti-rat Nrf2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. Immunoreactive proteins were visualized by using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (GE Healthcare Biosciences, Pittsburgh, PA, USA) and were exposed to Kodak X-OMAT film. The relative densities of Nrf2 and HO-1 bands were measured and quantified with NIH Image J. HO-1 was normalized against β-actin. The protein levels were expressed as percentage of control.

Quantitative real-time PCR

All of materials were from Life Technologies unless otherwise stated. Total RNA was extracted from cultured rat primary astrocytes by Trizol reagent according to the manufacturer’s instructions. cDNA was synthesized from 1 µg of total RNA in a 20 µl reaction mixture using a cDNA Reverse Transcription Kit with RNase Inhibitor. The mixture was incubated at 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min. The primers for GSTP1 were: forward, 5'-ACGCGAAAGCTTCAATTGTTGC-3' and reverse, 5'-GGTGGATGCGGAGCGCTAAAT-3', and the primers for hypoxanthine phosphoribosyltransferase-1 (an endogenous reference gene) were: forward, 5'-CCTTGCGACCTGCTGTATGTT-3' and reverse, 5'-GGTGGAGATGACCCAC-3'. Real-time PCR was carried out with IQ™ SYBR Supermix (Bio Rad, Hercules, CA, USA) in an Eppendorf Thermal Cycler. The reaction mixture was incubated at 95°C for 3 min and cycled 40 times from 95°C for 15 s to 60°C for 1 min.

Statistical analysis

Data from at least three independent experiments are presented as mean ± SEM and were analyzed statistically by t-test or one-way analysis of variance (ANOVA) followed by Dunnett post hoc test for comparisons to a single control or Tukey post hoc test
Fig. 2. Effect of LPNS on H₂O₂-induced release of lactate dehydrogenase (LDH) from cultured rat astrocytes. (A) Cells were pre-incubated for 24 h in the presence of different LPNS concentrations (0 control, 2.5, 5, 10 μg/ml) and then exposed for 4 h to 0.5 mM of H₂O₂ in FBS-free medium. LDH release was determined and expressed as a % of control. (B) LDH release from astrocyte cells pre-treated with 5 μg/ml LPNS for different durations (up to 48 h) before exposure to 0.5 mM H₂O₂. The results are expressed as a % of LDH release from vehicle-treated cells exposed to H₂O₂ (control). Values are means ± SE, n = 3 independent experiments. ANOVA followed by the Dunnett's test. * and ** indicate significant differences at the p < 0.05, p < 0.01 and p < 0.001 levels, respectively.

Fig. 3. Effect of LPNS on OGD-induced release of lactate dehydrogenase (LDH) from SH-SY5Y cells. Cells were pre-incubated for 24 h in media containing 0 (control), 2.5, 5 or 10 μg/ml LPNS and then underwent 6 h OGD and 24 h reoxygenation with glucose as described in the Methods. LDH release to media was then determined and expressed as a % of vehicle-treated cells exposed to OGD/reoxygenation. Values are means ± SE, n = 3 independent experiments. ANOVA followed by the Dunnett’s test. * and ** indicate significant differences at the p < 0.05, p < 0.001 levels, respectively.

for multiple comparisons between groups. A p value less than 0.05 was considered statistically significant ("p < 0.05", **p < 0.01 and ***p < 0.001). All analysis was performed with Prism 5.0 (Graph Pad, San Diego, CA, USA).

Results

Reduction in H₂O₂ and OGD/reoxygenation-induced cell death by LPNS

LDH release serves as an indicator of cell membrane damage and death. Pretreatment (24 h) with 5 and 10 μg/ml LPNS significantly reduced H₂O₂-induced LDH release in astrocytes compared to control by 23 ± 8% (p < 0.001) and 18 ± 2% (p < 0.01), respectively (Fig. 2A). 2.5 μg/ml LPNS did not show any protective effects. A time course indicated that pretreatment with 5 μg/ml LPNS for 12 and 24 h reduced LDH by 71 ± 14% (p < 0.01) and 44 ± 11% (p < 0.05), respectively (Fig. 2B).

LPNS also protected SH-SY5Y cells from the damage induced by OGD/reoxygenation. Thus, SH-SY5Y cells pretreated with 2.5 and 5 μg/ml LPNS for 24 h reduced the LDH release by 13 ± 6% (p < 0.05) and 78 ± 7% (p < 0.001), respectively (Fig. 3). There was no protection with 10 μg/ml pretreatment.

Inhibition of intracellular ROS production induced by H₂O₂

In order to investigate the mechanisms underlying LPNS-induced protection, the effects of LPNS on H₂O₂-induced intracellular ROS accumulation were examined. Astrocytes were labeled with DCFH-DA which forms fluorescent DCF upon oxidation with ROS. There was an increase in ROS production in astrocytes after exposure to 0.5 mM H₂O₂ for 1 h (2951 ± 92 vs. 2607 ± 10 in controls, p < 0.05; Fig. 4). That increase was significantly reduced in cells pretreated with 5 and 10 μg/ml LPNS for 24 h (2691 ± 149, p < 0.05, and 2561 ± 84, p < 0.001, respectively). The levels of ROS in cells pretreated with LPNS before H₂O₂ exposure were not significantly different from those found in control cells.

Upregulation of Nrf2 and its downstream antioxidant systems

Nrf2 plays a pivotal role in cellular defense against oxidative stress. Under normal conditions, Nrf2 is bound to a specific inhibitory protein, KEAP1, and is targeted for proteasomal degradation. However, under conditions of oxidative stress, Nrf2 is released
from KEAP1 and enters the nucleus where it upregulates antioxidant genes (Thimmulappa et al., 2002). Possible activation of Nrf2 by LPNS was investigated by Western blot. Results showed that nuclear Nrf2 levels in astrocytes after 1 h treatment with 5 μg/ml LPNS were 1.45 ± 0.14 fold higher than controls (p < 0.05, Fig. 5). Nrf2, on an enzyme that catalyzes the degradation of heme, is regulated by Nrf2. Pretreatment (24 h) with 5 μg/ml LPNS upregulated cellular HO-1 by 1.87 ± 0.34 fold in cultured primary astrocytes (p < 0.05, Fig. 6). GSTP1 is another downstream protein of Nrf2 and plays an important role in detoxification of various environmental toxins and oxidative stress. Real-time PCR was used to examine mRNA express of GSTP1 in astrocytes after pretreatment with 5 μg/ml LPNS and the results showed it was increased 231 ± 47% compared to control (p < 0.05, Fig. 7).

Discussion

This study examined the effects of total saponins in leaves of *Panax notoginseng* (LPNS) on oxidative stress-induced brain cell damage in vitro. In primary cortical astrocyte cells, LPNS reduced H₂O₂-induced cell damage. A similar protection was found in a neuronal cell line, SH-SYSY, with damage induced by OGD/reoxygenation. In primary astrocytes, the protective effects of LPNS were associated with reduced reactive oxygen species (ROS) levels, activation of Nrf2 and upregulation of downstream antioxidant mediators such as HO-1 and GSTP1. The present study demonstrates, for the first time, that LPNS exerts a protective effect on H₂O₂-induced astrocyte damage and OGD/reoxygenation-induced SH-SYSY cells injury by activating antioxidant systems. These effects may also protect the brain from damage induced by oxidative stress in neurological disorders.

Oxidative stress culminates from an imbalance between pro- and anti-oxidants and consequent excessive production of ROS. ROS have particularly deleterious effects on the nervous system because the brain is relatively deficient in antioxidant systems (Kedar, 2003). Hydrogen peroxide (H₂O₂), which is a byproduct of both normal and aberrant metabolism, has been shown to elicit cellular injury by initiating lipid peroxidation, protein oxidation and DNA damage (Cochrane, 1991). At higher concentrations (200–800 μM), H₂O₂ alters astrocyte membranes and the cytoskeleton (Zhu et al., 2005), which enable it to cross cell membranes and reach different subcellular compartments (de Almeida et al., 2008; Pedros et al., 2009). Inside the cell and in the presence of transition metal ions such as Fe²⁺ or Cu⁺, H₂O₂ can be converted into the highly reactive hydroxyl radical. H₂O₂ can induce mitochondrial calcium overload, mitochondrial membrane potential depolarization, and opening of the mitochondrial permeability transition pore, resulting in an increasing of the mitochondrial ROS formation (Copin et al., 2001; Jou et al., 2004). Thus, an excess of H₂O₂ induces imbalance in ROS generation and impairs cellular antioxidant defenses, leading finally to cell death, as shown in various cell types, including astrocytes (Blennert et al., 2006; Gyulkhandayan et al., 2003; Kaczero et al., 2010; Winterbourn, 1981). In the present study, LDH assay indicates pretreatment with LPNS significantly protected primary rat astrocytes from H₂O₂-induced cell damage and SH-SYSY cells from OGD/reoxygenation-induced damage. It has been reported that the water extract of the biennial flower of *P. notoginseng* showed effects in protecting neuronal PC12 cells against H₂O₂-induced cytotoxicity by reducing the ROS formation (Choi et al., 2010). In this study, we also found that H₂O₂ increased the production of ROS in cultured rat astrocytes and LPNS significantly inhibited ROS formation in a dose-dependent manner. This is likely responsible for the reduction in cell death induced by H₂O₂ in astrocytes and OGD, which is a model of ischemia and associated with free radical production in SH-SYSY cells (Guo et al., 2009). These results indicate that LPNS protected cells against the toxicity created by oxidative stress. It has been reported that LPNS (70–100 mg/kg) can reduce ischemic injury and have anti-depressive effects in animals, those diseases undergo the same basic pathology of oxidative damage (Fu et al., 2006; Xiang et al., 2011; Zhu et al., 2010).

Cells have evolved antioxidant defense mechanisms to neutralize ROS and maintain cellular redox homeostasis during oxidative stress. One of the most important cellular defense mechanisms against ROS and electrophilic intermediates is mediated through the antioxidant responsive element (ARE). ARE-dependent cellular defense systems are regulated by a transcription factor, Nrf2 (Jaiswal, 2004; Mathers et al., 2004), which is held in the cytoplasm by a cytoskeletal-associated specific inhibitory protein KEAP1 under conditions of normal cellular redox state and is continuously targeted to proteasomal degradation. Under conditions
of oxidative stress, Nrf2 dissociates from KEAP1 and then translocates to the nucleus, binds to ARE to induce antioxidant and phase II detoxification enzymes such as HO-1 and GSTP1 (Kobayashi and Yamamoto, 2005; Park et al., 2011). There is increasing evidence that the Nrf2-ARE pathway is involved in neurodegenerative disease and the expression of ARE driven genes such as NQO1 and HO-1 is increased in post mortem brain tissue from PD patients (Park et al., 2011; Schipper et al., 1998; Yoo et al., 2003). These changes could be a neuroprotective response mediated by Nrf2 activation. Induction of Nrf2-mediated transcription, particularly in astrocytes, has been shown to protect against neurotoxicity from a variety of insults (Calkins et al., 2005). Consistent with those reports, the present study revealed Nrf2 accumulation in the nucleus and upregulation of downstream antioxidant enzymes (HO-1, GSTP1) after treatment with LPNS in astrocytes. As ROS levels are tightly controlled by an inducible antioxidant program that responds to cellular stressors and is predominantly regulated by Nrf2 (DeNicola et al., 2011), LPNS may increase the transcription of Nrf2 to stably elevate the basal Nrf2 antioxidant program and thereby reducing intracellular ROS.

Oxidative stress is an important molecular mediator of astrocyte injury in different neurological conditions and may be an important therapeutic target. One strategy for detoxifying different reactive oxygen and nitrogen species that are produced in such conditions is induction of the Phase II gene response by the use of chemicals or conditions that promote the translocation of Nrf2 from the cytosol to the nucleus, where it binds to genomic antioxidant response elements. The present study examined the hypothesis that pretreatment with LPNS would stimulate antioxidative responses in astrocytes and neurons, and would mitigate the effects of ROS-mediated neurotoxicity via the Nrf2 pathway. We demonstrate that LPNS, a potent stimulator of antioxidant responses, was effective in reducing neurotoxicity associated with decreasing H2O2-induced LDH release, decreasing ROS production and upregulating HO-1 and GSTP1 expression through the activation of Nrf2 in astrocytes. Data support a key role for astrocyte-produced antioxidants in modulating oxidative stress and neuronal damage in response to H2O2 and other forms of oxidative damage (Dringen and Hirrlinger, 2003). LPNS may, therefore, promote neuronal survival through activating the astrocyte-mediated antioxidative pathway by decreasing the amount of ROS and increasing the activities of antioxidative enzymes.

In summary, LPNS has significant protective effects against cell damage induced by H2O2 and OGD/reoxygenation. It inhibits ROS by activating Nrf2 and downstream antioxidant systems. Moreover, this study supports the hypothesis that LPNS stimulates antioxidative responses in astrocytes, as well as neurons, thereby attenuating the effects of ROS-mediated neurotoxicity. This is the first report on the therapeutic effects of LPNS on oxidative damage after H2O2 or OGD/reoxygenation insult and the role of LPNS in regulating ROS and oxidative stress response in brain cells. All of these indicate that LPNS could be a potential therapeutic agent to ameliorate oxidative damage and neurodegenerative processes.

Conflict of interest

The authors declare that they have no conflict of interest.

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