NEUROPROTECTIVE EFFECT OF ANDROGRAPHOLIDE AGAINST ISCHEMIC STROKE IN RATS THROUGH REDUCING INOS AND GP91PHOX/NOX2 EXPRESSION

Yu-Chang Hou2,6,7, Yea-Hwey Wang4, Kuo-Tong Liou3, Yuh-Chiang Shen1,5,*

1National Research Institute of Chinese Medicine, Taipei, Taiwan
2Department of Chinese Medicine, Taoyuan General Hospital, Department of Health, Taoyuan, Taiwan
3Department of Chinese Martial Arts, Chinese Culture University, Taipei, Taiwan
4Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan
5Institute of Biomedical Sciences, National Chung-Hsing University, Taichung, Taiwan
6Department of Nursing, Yuanpei University, Hsinchu, Taiwan
7Department of Bioscience Technology, Chuan-Yuan Christian University, Taoyuan, Taiwan

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Andrographolide, an active component isolated from Andrographis paniculata, has been reported to exhibit anti-inflammatory effects in neutrophils and microglial cells, but its effects on central nervous system (CNS) are unclear. In this study, we explored the CNS protective effect of andrographolide in an ischemic stroke animal model. We performed a cerebral ischemic/reperfusion (CI/R) injury in rats to study whether treatment of andrographolide (5-10 μg/kg, i.v.) 1 h after ischemia could protect rats against stroke. Treatment with andrographolide dose-dependently ameliorated CI/R-induced brain infarction (reduced by 32%-51%) and improved neurological deficits in rats at day 1 after stroke. Elevated pathophysiological markers including reactive oxygen species (ROS) production and protein nitrosylation were significantly reduced by andrographolide. CI/R dramatically enhanced expression of inducible nitric oxide synthase (iNOS), gp91phox/NADPH oxidase 2 (NOX2) and interleukin-1β (IL-1β) that paralleled to the activation of nuclear factor-kappa B (NF-κB) and hypoxia-inducing factor 1-alpha (HIF-1α), and all were significantly diminished by andrographolide. Andrographolide compromises CI/R induced expression of iNOS and gp91phox/NOX2 through impairing NF-κB and HIF-1α activation that confers it as a potential ischemic stroke therapeutic agent.

Key words: andrographolide, CI/R, HIF-1α, iNOS, NF-xB, gp91phox/NADPH oxidase 2

Introduction

Ischemic stroke is ranked as the third most common causes of death in developed countries, although the recent decline in death rates has been reported1. It has been well documented that...
“excitotoxicity” is the major pathophysiological mechanism underlying ischemic stroke-induced brain injury, in which an inappropriate activation of ionotropic N-methyl-D-aspartate receptors in the brain by excessive glutamate accumulation in the extracellular space under stroke onset.\(^2\) Excessive amount of glutamate excites neurons to death through inducing overproduction of reactive oxygen species (ROS) such as hydroxyl radicals (OH\(^-\)), superoxide anions (O\(_2\)\(^-\)) and hydrogen peroxide (H\(_2\)O\(_2\)), and reactive nitrogen species such as nitric oxide (NO\(^-\)) and peroxynitrite (OONO\(^-\)), the so-called oxidative and/or nitrosative stresses.\(^2\) The main free radical producing-enzyme systems involved include mitochondria, cyclooxygenase (COX), xanthine oxidase, gp91\(^{phox}\)/NADPH oxidase 2 (NOX2), and inducible-nitric oxide synthase (iNOS) in response to the activation of proinflammatory mediators (e.g., interleukin-1\(\beta\), IL-1\(\beta\)), produced by recruited leukocytes (e.g., neutrophils, macrophages and T-cells), active microglial cells, damaged neurons and astrocytes in ischemic stroke-damaged tissues.\(^2,3\)

Enormous production of free radicals as mentioned above damages tissues through breaking DNA or denaturing protein and lipid by inducing protein nitrosylation and malondialdehyde formation of the cell membrane and organelles.\(^2,3\) An inflammatory cascade is consequently activated in damaged tissue leading to leukocyte infiltration, expression of iNOS, gp91\(^{phox}\)/NOX2, and release of proinflammatory cytokines for amplification of inflammatory responses.\(^4\) Moreover, activation of transcriptional factor(s), e.g., nuclear factor-kappa B (NF-\(\kappa\)B), plays a pivotal role in mediating oxidative stress-induced cell injury and in regulating post-ischemic inflammation, possibly through upregulation of inflammatory genes and proteins that contribute to cell death in cerebral ischemia.\(^5,6\)

We have previously reported that andrographolide (Fig. 1) displays potent anti-inflammatory effect by diminishing oxygen radical production and Mac-1 up-expression in human neutrophils through a PKC-dependent pathway,\(^7,8\) and is beneficial in rats suffering with endotoxaemia by inhibiting the NO production \(\text{via}\) reducing the expression of iNOS,\(^7,9\) possibly through modulation of NF-\(\kappa\)B activation.\(^10\) The protecting effect of andrographolide on site of central nerve system (CNS) and the possible underlying mechanisms of action are still unclear.

In current work, we established an animal model of ischemic stroke to elucidate whether treatment with andrographolide 1 h after ischemia could protect rat against cerebral ischemic/reperfusion (CI/R) injury. In particular, the molecular mechanism(s) of action involved in the ameliorative effect of andrographolide was elucidated.
Materials and Methods

Animals and induction of cerebral ischemic-reperfusion injury

All animal procedures and protocols were performed in accordance with The Guide for the Care and Use of Laboratory Animals (NIH publication, 85-23, revised 1996) and were reviewed and approved by the Animal Research Committee at National Research Institute of Chinese Medicine. The CI/R model in rats has previously been described.2 Briefly, male adult Sprague-Dawley rats weighing 250–350 g (National Laboratory Animal Breeding and Research Center, Taipei, Taiwan) were used. Rats were anesthetized with chloral hydrate (0.4 g/kg, i.p.). The right femoral artery was cannulated for continuous monitoring of the heart rate and blood pressure. The right middle cerebral artery (RMCA) was ligated. Immediately after occlusion of the RMCA, both common carotid arteries (CCA) were clipped for 1 h to produce a cortical infarction before drug(s) treatment. Twenty-four hours after the surgery, the rats were anesthetized and sacrificed. During the entire experiment, the blood gas data and physiological data were monitored as in our previous report.2 The experimental grouping included one sham-operated group (sham, n=10), two andrographolide (5 or 10 µg/kg)-treated groups (n=10, for each dose), one positive control N\textsuperscript{G}-Nitro-L-arginine methyl ester (L-NAME) (100 µg/kg)-treated group and one vehicle-control CI/R group (CI/R only, n=10). Sham-operated rats underwent the same surgical procedures without RMCA occlusion and CCA clipping. Additional animals from the groups described above were used for other assays including immunoblotting and immunohistochemistry staining. Dihydroethidium (DHE, Molecular Probes, Eugene, OR, USA; 2.0 mg in 200 µl normal saline) was given i.v. to rats just before the onset of reperfusion for determination of free radical production.

Drug administration

Drug solutions (0.3 ml) were administered intravenously (i.v.) via the right femoral 1h after RMCA occlusion at two different doses (5 or 10 µg/kg). Andrographolide (Sigma-Aldrich, St. Louis, MO, USA) was first dissolved in 50 µl of ethanol to make a stock solution of 1.0 mg/ml and were diluted with normal saline to final concentrations (5-10 µg/ml). Rats injected with 0.3 ml of normal saline with 0.01% of ethanol were used as a vehicle control in the CI/R only and sham-operated groups.

Evaluation of infarct volume and neurological deficits after CI/R injury

Twenty-four hours after reperfusion, rats were sacrificed by rapid decapitation under deep anesthesia. The whole brain was rapidly removed and sliced into 2-mm-thick coronal sections for staining with 2,3,5-triphenyltetrazoliumchloride (Sigma-Aldrich, USA). The slices were photographed with a digital camera and analyzed by an image processing system (AlphaEaseFC 4.0, Alpha Innotech, San Leandro, CA, USA). Infarct volume was obtained according to the indirect method and corrected for edema (Shen et al., 2008). The infarct volume was expressed as a percentage (%) of the whole brain volume. The neurobehavioral study of rats was carried out just before the sacrifice as described by Garcia et al.11 with some modifications.2 Each rat was scored by three examiners who had no knowledge of the procedure that the rat had undergone.
**Immunohistochemical staining**

The brain samples were prepared for immunohistochemical staining as described. The tissue slices mounted on the coverslips were randomly selected for incubation with appropriate first antibodies against nitrotyrosine (NT) (1:50, Upstate, Lake Placid, NY, USA), NOX2 (1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA), iNOS (1:50), IL-1β (1:50), p65NF-κB (1:50) and HIF-1α (1:50) (all from BD PharMingen, San Diego, California, USA) in phosphate-buffered saline (PBS) containing 3% albumin at 4 °C overnight. After washing, sections were incubated with fluorescein isothiocyanate (FITC)- or phycoerythrin-Cy5 (PE-Cy5)-conjugated second antibodies. All coverslips were mounted with mounting medium containing a proper dilution of 4',6-diamidino-2-phenylindole (DAPI) to counterstain DNA in the nuclei. The sliced tissues were examined using a laser-scanning confocal microscope (Leica DM IRBE microscope, TCS SPII confocal scanner; Leica Microsystems, Heidelberg, Germany). The distribution and numbers of immunopositively stained cells were determined, and averaged in the entire field of the image (280 × 280 μm) after they had randomly been taken from six different non-overlapping regions sampled in the ischemic region under high magnification (×40 or ×63 objective).

**Western immunoblot analysis**

Equal amounts of protein prepared as described before were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to a hydrophobic polyvinylidene difluoride (PVDF) membrane. After blocking with 5% nonfat milk, the membrane was washed and incubated overnight at 4 °C with an antibody against NOX2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), p65NF-κB (BD Transduction Laboratories, BD Biosciences, San Diego, CA, USA), iNOS (BD Bioscience Pharmingen, San Diego, CA, USA), HIF-1α (Novus Biologicals, Littleton, CO, USA), or β-actin (Sigma-Aldrich, St Louis, MO, USA) at a properly titrated dilution (1:200–2500). After additional washes, the membrane was incubated with a properly titrated second antibody. The immunoblot on the membrane was visible after development with an enhanced chemiluminescence (ECL) system (Perkin-Elmer, Wellesley, MA, USA) and was quantitated using an image program (Multi Gauge v2.2 software, Fujifilm, Tokyo, Japan).

**Statistical analysis**

All data in the text, tables, and figures are given as the mean±S.E.M. (standard error of the mean). Data were analyzed by a two-tailed t-test or one-way analysis of variance (ANOVA) followed by the post hoc Student-Newman-Keuls (S-N-K) t-test for multiple comparisons. The dose dependence of each drug was analyzed by a simple linear regression analysis of response levels against dose of the drug and tested the slope of the regression line against 0 by Student’s t-test. Values of p<0.05 were considered significant.

**Results**

**Amelioration of CI/R-induced cerebral infarction and neurological deficits by andrographolide**

In this study, CI/R injury (stroke) induced remarkable cerebral infarction to around 13.0% of the whole brain. Treatment of andrographolide (5
and 10 μg/kg, i.v.) 1 h after ischemia dose-dependently reduced the CI/R-induced brain infarction by 32%~51% (Table 1, one-way ANOVA, \( p<0.05, n=10 \) for each group). Treatment with L-NAME (100 μg/kg, i.v.), a non-specific NOS inhibitor also reduced brain infarction by 32% (one-way ANOVA followed by S-N-K test, \( p<0.05, n=10 \) for each group). The blood gas data (pH, \( p_{CO_2} \), and \( p_{O_2} \)) and physiological data (blood pressure and heart rate) were all monitored, and no significant difference could be observed among these groups (data not shown). In parallel with the cerebral infarction, CI/R injury also induced severe neurological deficits as compared to that of sham-operated rats (Table 1; one-way ANOVA followed by S-N-K t-test, \( p<0.05, n=10 \) for each group). Treatment with andrographolide (5 and 10 μg/kg) and L-NAME both significantly reversed CI/R-induced neurological deficits (Table 1; one-way ANOVA followed by S-N-K t-test, \( p<0.05, n=10 \) for each group).

### Reduction of CI/R-induced oxidative/nitrosative tissue damage by andrographolide

CI/R induced a significant increase in oxidative/nitrosative-mediated tissue damage as determined by protein tyrosine nitrosylation (nitrotyrosine, NT) at 24 h after ischemic injury (Fig. 2). Formation of nitrotyrosine colocalized with the production of superoxide anion in the damaged tissues which is revealed by intensive staining of dihydroethidine (DHE) (Fig. 2). Andrographolide extensively reduced CI/R-induced nitrotyrosine formation (Fig. 2, one-way ANOVA, \( p<0.05, n=5 \) for each group), as well as superoxide anion production (DHE staining) in the damaged tissues (Fig. 2, one-way ANOVA, \( p<0.05, n=5 \) for each group).

### Effect of andrographolide on CI/R-induced gp91phox/NOX2, iNOS expression and IL-1β production in DHE positive staining cells

Expression of gp91phox/NOX2 (Fig. 3 and Fig. 8), iNOS (Fig. 4 and Fig. 8), and IL-1β (Fig. 5) proteins was upregulated by CI/R injury at 24 h after ischemia. Treatment with andrographolide (5 and 10 μg/kg, i.v.) significantly reduced the immunoreactivity of gp91phox/NOX2, iNOS and IL-1β (Fig. 3, Fig. 4, Fig. 5, and Fig. 8, one-way ANOVA, \( p<0.05, n=5 \) for each group). Moreover, we found that the expression of gp91phox/NOX2, iNOS and IL-1β were all colocalized with the DHE staining, a marker for inflammation-associated oxidative stress (Fig. 3, Fig. 4, and Fig. 5), and the DHE staining alone was also significantly diminished by andrographolide (Fig. 3, Fig. 4, and Fig. 5, one-way ANOVA, \( p<0.05, n=5 \) for each group), indicating that CI/R-induced activation and accumulation of oxidative stress, and the

### Table 1. Effects of andrographolide on cerebral infarction and neurological deficits in cerebral ischemic reperfusion (CI/R) injured rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Infarction (%</th>
<th>Neurological deficits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>N.A.</td>
<td>12.0±0*</td>
</tr>
<tr>
<td>CI/R (stroke)</td>
<td>13.0±0.3</td>
<td>3.0±1.0</td>
</tr>
<tr>
<td>+ andrographolide (5 μg/kg)</td>
<td>8.8±0.5*</td>
<td>5.5±0.5*</td>
</tr>
<tr>
<td>+ andrographolide (10 μg/kg)</td>
<td>6.4±0.3*</td>
<td>6.8±0.6*</td>
</tr>
<tr>
<td>+ L-NAME (100 μg/kg)</td>
<td>8.9±0.7*</td>
<td>5.6±0.3*</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M. (\( n=10 \) for each data point). *\( p<0.05 \), compared with the corresponding CI/R (stroke) only group, respectively, by one-way ANOVA followed by post-hoc Student-Newman-Keuls (S-N-K) for multiple comparison. N.A., data not available.
Fig. 2. Effects of andrographolide on changes in nitrotyrosine (NT) and superoxide formation at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. Upper panel, confocal images of NT formation (green) and a superoxide marker (oxidized dihydroethidium (DHE, red)) in the ipsilateral cerebral cortex. Arrows indicate the colocalization (yellow) of green fluorescence and red fluorescence (DHE) in the merged columns. Treatment with andrographolide (5 or 10 µg/kg; i.v.; CI/R+AND5 or CI/R+AND10) significantly reduced the staining of NT and DHE. Lower panel, statistical results from five independent experiments were calculated as the mean±S.E.M. for each data point. * p<0.05, compared with the corresponding CI/R group only by one-way ANOVA followed by Student-Newman-Keuls t-test. N.D., not detectable.

Fig. 3. Effects of andrographolide on changes in NOX2 and superoxide production at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. Upper panel, confocal images of NOX2 expression (green) and superoxide marker (DHE, red) in the ipsilateral cerebral cortex. The nuclei of these cells were visualized by DAPI staining (blue). Arrows indicate the colocalization (yellow) of green fluorescence and red fluorescence in the merged columns. Treatment with andrographolide (5 or 10 µg/kg; i.v.; CI/R+AND5 or CI/R+AND10) significantly reduced the staining of NOX2 and superoxide. Lower panel, statistical results from five independent experiments were calculated as the mean ± S.E.M. for each data point. * p<0.05, compared with the corresponding CI/R group only by one-way ANOVA followed by Student-Newman-Keuls t-test. N.D., not detectable.
Fig. 4. Effects of andrographolide on changes in iNOS and superoxide production at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. Upper panel, confocal images of iNOS expression (green) and superoxide production (DHE, red) in the ipsilateral cerebral cortex. Arrows indicate the colocalization (yellow) of green fluorescence and red fluorescence in the merged columns. Treatment with andrographolide (5 or 10 μg/kg; i.v.; CI/R+AND5 or CI/R+AND10) significantly reduced the staining of iNOS and superoxide production. Lower panel, statistical results from five independent experiments were calculated as the mean ± S.E.M. for each data point. * p<0.05, compared with the corresponding CI/R only group by one-way ANOVA followed by Student-Newman-Keuls t-test. N.D., not detectable.

Fig. 5. Effects of andrographolide on changes in IL-1β and superoxide production at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. Upper panel, confocal images of IL-1β production (green) and superoxide production (DHE, red) in the ipsilateral cerebral cortex. Arrows indicate the colocalization (yellow) of green fluorescence and red fluorescence in the merged columns. Treatment with andrographolide (5 or 10 μg/kg; i.v.; CI/R+AND5 or CI/R+AND10) significantly reduced the staining of IL-1β and superoxide production. Lower panel, statistical results from five independent experiments were calculated as the mean ± S.E.M. for each data point. * p<0.05, compared with the corresponding CI/R only group by one-way ANOVA followed by Student-Newman-Keuls t-test. N.D., not detectable.
Fig. 6. Effect of andrographolide on changes in NF-κB upexpression at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. Upper panel, confocal images of p65NF-κB upexpression (green) in the ipsilateral cerebral cortex. The nuclei of these cells were visualized by DAPI staining (blue). Arrows indicate the translocation of p65NF-κB to nuclei. Treatment with andrographolide (5 or 10 µg/kg; i.v.; CI/R+AND5 or CI/R+AND10) significantly reduced the staining and nuclear translocation of p65NF-κB. Lower panel, statistical results from five independent experiments were calculated as the mean ± S.E.M. for each data point. *p<0.05, compared with the corresponding CI/R only group by one-way ANOVA followed by Student-Newman-Keuls t-test. N.D., not detectable.

Fig. 7. Effect of andrographolide on changes in HIF-1α and superoxide production at 24 h after cerebral ischemic reperfusion (CI/R) injury in rats. Upper panel, confocal images of HIF-1α upexpression (green) and superoxide production (DHE, red) in the ipsilateral cerebral cortex. Arrows indicate the translocation of HIF-1α to nuclei. Treatment with andrographolide (5 or 10 µg/kg; i.v.; CI/R+AND5 or CI/R+AND10) significantly reduced the staining and nuclear translocation of HIF-1α. Lower panel, statistical results from five independent experiments were calculated as the mean ± S.E.M. for each data point. *p<0.05, compared with the corresponding CI/R only group by one-way ANOVA followed by Student-Newman-Keuls t-test. N.D., not detectable.
upregulation of two prooxidative enzymes gp91phox/NOX2, iNOS and proinflammatory cytokine IL-1β in inflammatory cells were all limited by andrographolide.

Effect of andrographolide on CI/R-induced activation of p65NF-κB and HIF-1α

Ischemic stroke induced substantial upexpression and activation/nuclear translocation of p65NF-κB (Fig. 6) and HIF-1α (Fig. 7), and were both significantly diminished by andrographolide treatment (Fig. 6, Fig. 7 and Fig. 8, one-way ANOVA, $p<0.05$, $n=5$ for each group), indicating that proinflammatory and hypoxic transcriptional factors including p65NF-κB and HIF-1α were both modulated by andrographolide (Fig. 8, one-way ANOVA, $p<0.05$, $n=5$ for each group).

Discussion

Andrographolide has been reported to protect cardiomyocytes against hypoxia/reoxygenation injury, but its effect on CNS is unclear. Herein, our results demonstrate for the first time that treatment of andrographolide at pharmacological applicable range (5-10 μg/kg, i.v.) 1 h after ischemia can ameliorate CI/R injury (brain infarction and neurological deficits) in rats by diminishing massive ROS production in the brain and inhibiting hypoxic and proinflammatory responses, through reducing expression of iNOS and gp91phox/NOX2 via impairing NF-κB and HIF-1α activation.

In this study, andrographolide effectively ameliorated CI/R-induced brain damage by reducing protein nitrosylation induced by peroxynitrite (ONOO$^-$), a product of the reaction of NO and
Andrographolide ameliorates stroke in rats

Superoxide anion possibly generated from iNOS and gp91phox/NOX2 in oxidative (DHE) related positive staining cells, most likely the inflammatory cells, indicating that targeting inflammatory cells plays a major role in mediating andrographolide’s protective effect. These could be further supported by previous reports, demonstrating that a non-specific NOS inhibitor (L-NAME) and a specific NOX inhibitor (apocynin) both displayed potent anti-oxidative/nitrosative capacity and effectively reduced stroke injury respectively. Other studies have emphasized a dual-key mechanism, whereby microglial gp91phox/NOX2 or glial iNOS activation alone is relatively mild, but if activated simultaneously are synergistic in killing neurons or inducing cell death during hypoxia, through generating peroxynitrite. Therefore, it is reasonable to observe that andrographolide, with dual potential in the inhibition of iNOS and gp91phox/NOX2 expression, is more powerful than single iNOS inhibitor (L-NAME) to simultaneously diminish NO and ROS production, in turn, lessening the peroxynitrite-mediated amplification of CI/R injury. In contrast, andrographolide did not directly modulate the gp91phox/NOX2 activity and did not show free radical scavenging activity (data not shown). Whether the NOS activity could be directly inhibited by andrographolide needs further investigation.

Moreover, proinflammatory cytokines (e.g., IL-1β) have been reported to upregulate adhesion molecules (e.g., intercellular adhesion molecule-1) during CI/R injury for mediating recruitment of inflammatory cells. Here, we found that the upexpression of IL-1β was colocalized with DHE positive staining cells (most likely microglial cells and leukocytes) and was reduced by andrographolide treatment. This may partially account for the suppression of inflammatory cells (DHE positive stained cells) activation/accumulation and limitation of inflammation by andrographolide in the infarction area. Since most of these inflammatory-related proteins (e.g., IL-1β and iNOS) are down stream gene products of transcriptional factors, particularly NF-κB, which is activated during oxidative and/or inflammatory stress, we found that andrographolide treatment decreased the activation/nuclear translocation of NF-κB in CI/R-injured rat brain, indicating that andrographolide could suppress NO production and inflammatory-related proteins via the NF-κB-dependent mechanism rather than by direct interfering with NOS activity. This suggestion could be further supported by other’s reports demonstrating that andrographolide could inhibit expression and transactivation of NF-κB in inflammatory cells.

In addition to NF-κB, up-regulation of HIF-1α during hypoxia also has been reported to enhance iNOS expression in activated microglial cell in the brain and through which to promote neuronal injury. Here we demonstrated for the first time that andrographolide could inhibit the activation of NF-κB and HIF-1α, as well as the expression of iNOS and gp91phox/NOX2 induced by CI/R. These results suggested activation of NF-κB and HIF-1α are responsible for the upexpression of iNOS and gp91phox/NOX2 under hypoxia. Andrographolide has been recently reported to regulate macrophage activation and polarization through modulating the PI3-kinase/Akt signaling pathway. Similarly, our data showed that andrographolide, probably like a PI3-kinase inhibitor, can inhibit CI/R-induced pAkt expression (Fig. 8), activation of NF-κB and HIF-1α, and the expression of iNOS and gp91phox/NOX2. These
results suggested that the PI3-kinase/Akt dependent activation of NF-κB and HIF-1α could be responsible for the upexpression of iNOS and gp91phox/NOX2 under hypoxia, however, the relationship(s) between the PI3-kinase/Akt dependent for NF-κB- and/or HIF-1α-associated iNOS and/or gp91phox/NOX2 activation is still unclear in detail and is need further study.

In conclusion, our results demonstrate, for the first time, that andrographolide significantly ameliorates CI/R-induced rat brain damage by reducing ROS and NO production, as well as protein nitrosylation via limiting the expression of inflammation-related prooxidative enzymes (i.e., gp91phox/NOX2 and iNOS) and the production of proinflammatory cytokines (e.g., IL-1β) in injured tissue by, at least in part, reducing activation of NF-κB and HIF-1α. As a potent anti-inflammatory drug and neuroprotective agent, whether andrographolide could be beneficial in acute ischemic stroke in humans deserves further investigation and clinical evidence.

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穿心蓮乙素 (andrographolide) 經由抑制iNOS和gp91phox/NADPH oxidase 2 (NOX2) 的表現
保護缺血型中風大鼠腦組織

侯毓昌 2,6,7、王雅惠 4、劉國同 3、沈郁強 1,5,*

1 國立中國醫藥研究所，台北，台灣
2 衛生署桃園醫院中醫科，桃園，台灣
3 中國文化大學國術系及運動教練研究所，台北，台灣
4 台北榮民總醫院精神部，台北，台灣
5 中興大學生物醫學研究所，台中，台灣
6 元培科技大學護理學系，新竹，台灣
7 中原大學生物科技系，桃園，台灣

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穿心蓮乙素 (andrographolide) 曾被報導具有抗發炎細胞（嗜中性白血球及樹膠細胞）活
化作用，但其對中樞神經系統的保護作用並不清楚。本研究以大鼠腦部缺血再灌流（ischemic/
reperfusion，CI/R）引發腦中風（ischemic stroke）模式探討穿心蓮乙素之中樞保護作用。大
鼠中風後1小時給予穿心蓮乙素（5-10 μg/kg，i.v.），並於中風後24小時評估，可以依劑量相
關的方式減少腦梗塞區域（infarction），並顯著改善大鼠之運動神經行為能力（neurological
deficits）；中風鼠腦部主要病理生化指標如自由基（ROS）、硝化蛋白質（nitrotyrosine）也都
顯著被穿心蓮乙素抑制。中風也造成病理因子iNOS，gp91phox/NADPH oxidase 2（NOX2）及
IL-1β大量表現，且與轉錄因子NF-κB及HIF-1α之大量表現平行，而這些現象都因於給予穿心蓮
乙素而顯著下降；因此我們推測穿心蓮乙素可經由抑制NF-κB及HIF-1α而轉錄因子的活化減少
中風造成的大量自由基、iNOS、NOX2及IL-1β表現；達到中樞神經保護效果，因此穿心蓮乙
素應有潛力發展成為治療缺血型中風的神經保護藥物。

關鍵字：穿心蓮乙素、缺血再灌流、HIF-1α、iNOS、NF-κB、NOX2

*聯絡人：沈郁強 國立中國醫藥研究所 112台北市北投區國立農業二路45-1號 電話：02-28201999分機9101
傳真：02-28264266 電子郵件信箱：yuhcs@nrim.edu.tw