Supporting Information

Andrographolide inhibits PI3K/AKT-dependent NOX2 and iNOS expression protecting mice against hypoxia/ischemia-induced oxidative brain injury

Chang-Ming Chern³,*, Kuo-Tong Liou⁶,*, Yea-Hwey Wang⁵,⁷, Jyh-Fei Liao¹, Jiin-Cherng Yen¹, Yuh-Chiang Shen²,⁴

Affiliation

¹ Department of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan
² National Research Institute of Chinese Medicine, Taipei, Taiwan
³ Division of Neurovascular Disease, Neurological Institute, Taipei Veterans General Hospital and School of Medicine, National Yang-Ming University, Taipei, Taiwan
⁴ Institute of Biomedical Sciences, National Chung-Hsing University, Taichung, Taiwan
⁵ Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan
⁶ Department of Chinese Martial Arts, Chinese Culture University, Taipei, Taiwan
⁷ Department of Nursing, College of Medicine and Nursing, Hungkuang University, Taichung, Taiwan.

Correspondence

Dr Yuh-Chiang Shen
National Research Institute of Chinese Medicine
155-1 Li-Nung Street
Sec. 2, Shih-Pai
Taipei 112
Taiwan
Phone: +886/2/28201999 ext. 9101
Fax: +886/2/28264266
yuhcs@nricm.edu.tw

Dr Jiin-Cherng Yen
Department of Pharmacology, School of Medicine
National Yang-Ming University
155 Li-Nung Street
Sec. 2, Shih-Pai
Taipei 112
Taiwan
Phone: +886/2/28267090
Fax: +886/2/28231521
jcyen@ym.edu.tw

*These authors contributed equally to this study.
### Table 1S Physiological measurements before and after right middle cerebral artery occlusion.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham CI/R</th>
<th>CI/R+AND (100 μg/kg)</th>
<th>CI/R+AND (10 μg/kg)</th>
<th>CI/R+DPI (1 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>before MCAO 7.38±0.01</td>
<td>7.36±0.02</td>
<td>7.33±0.02</td>
<td>7.32±0.01</td>
</tr>
<tr>
<td></td>
<td>after MCAO 7.42±0.02</td>
<td>7.40±0.02</td>
<td>7.41±0.01</td>
<td>7.41±0.02</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>before MCAO 158±6</td>
<td>156±7</td>
<td>153±5</td>
<td>158±8</td>
</tr>
<tr>
<td></td>
<td>after MCAO 160±4</td>
<td>158±6</td>
<td>142±3</td>
<td>141±5</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>before MCAO 35±2</td>
<td>36±3</td>
<td>34±4</td>
<td>38±4</td>
</tr>
<tr>
<td></td>
<td>after MCAO 38±3</td>
<td>35±2</td>
<td>39±3</td>
<td>36±3</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>before MCAO 88±1</td>
<td>94±2</td>
<td>91±2</td>
<td>90±2</td>
</tr>
<tr>
<td></td>
<td>after MCAO 89±2</td>
<td>92±1</td>
<td>86±1</td>
<td>93±1</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>before MCAO 535±23</td>
<td>528±28</td>
<td>521±24</td>
<td>525±21</td>
</tr>
<tr>
<td></td>
<td>after MCAO 529±25</td>
<td>515±27</td>
<td>509±25</td>
<td>517±23</td>
</tr>
</tbody>
</table>

Sham, sham-operated; CI/R, cerebral ischemia/reperfusion; CI/R+AND, cerebral ischemia/reperfusion plus andrographolide; CI/R+DPI, cerebral ischemia/reperfusion plus diphenyleneiodonium; MCAO, right middle cerebral artery occlusion; MABP, mean arterial blood pressure; HR, heart rate; bpm, beats per min; Data are presented as mean±SEM.
Fig. 1S Chemical structure of andrographolide.
**Fig. 2S** Working model for the ischemic injury protective effect of andrographolide.
**Fig. 3S** Effects of andrographolide on changes in pAKT up-expression at 24 h after cerebral ischemic/reperfusion (CI/R) injury in mice.

Confocal images of pAKT up-expression in the ipsilateral peri-infarct region. The nuclei of these cells were visualized by DAPI staining (blue). Arrows indicate the up-expression of pAKT.