Neuroprotective action of *Astragalus mongholicus* aqueous extract in experimental rats suffering from spinal cord injury

Yan Zhi-Qin*, Bao Shao-Zhi, Zhang Shun-Kai and Yu Jun-Ru

Department of Neurology, The Third Affiliated Hospital of Wenzhou Medical College, Wenzhou, Zhejiang Province, China.

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*Astragalus mongholicus* aqueous extract (AMAE) is widely used for its medicinal properties. In the present study, we evaluated the role of AMAE on spinal cord lipid peroxidation (LPO), antioxidant status, apoptosis index and inducible nitric oxide synthases (iNOS) positive expression rate in rats. Male albino rats of Wistar strain were divided into four groups: Group I, normal control rats; Group II, model rats suffering from spinal cord injury (SCI); Group III and V, AMAE rats supplemented with AMAE. Results showed that increased spinal cord LPO, apoptosis index, iNOS positive expression rate and decreased antioxidant enzymes activities were observed in model rats (Group II). Administration of AMAE significantly dose-dependently decreased spinal cord LPO, apoptosis index, iNOS positive expression rate and increased antioxidant enzymes activities in AMAE rats. These findings demonstrated that AMAE can enhance the antioxidant status and decrease the incidence of free radical-induced LPO, and display strong neuroprotective activity in the experimental rats.

Key words: *Astragalus mongholicus* aqueous extract, rat, spinal cord injury, inducible nitric oxide synthases (iNOS) positive expression rate.

INTRODUCTION

Spinal cord injury (SCI) produces a secondary protracted wave of oligodendrocyte death in degenerating white-matter tracts distant from the injury site for weeks after the initial event (Crowe et al., 1997; Liu et al., 1997; Shuman et al., 1997; Abe et al., 1999; Li et al., 1999; Springer et al., 1999; Casha et al., 2001). When judged primarily by anatomical criteria (Beattie et al., 2000), the predominant type of oligodendrocyte death appears to be apoptosis. Recent studies have used a variety of immortalized, engineered, or isolated rodent-derived precursor stem cells transplanted into rodent models of SCI. Many of these studies focused on cell survival and did not address differentiation, functional recovery or the causal relationship between successful engraftment and observed behavioral improvements.

*Astragalus mongholicus* has been used as one of the primary Mongolian and Chinese tonic herbs for thousands of years. In modern Chinese medicine, *A. mongholicus* (Huangqi) is widely used as an immune modulator, especially to support immune health for various chronic degenerative diseases. Reports indicate that the main actions include anxiolytic, antidepressant, antiinflammatory and antiaggressive effects (Molodavkin et al., 2000). *A. mongholicus* is an important candidate for the treatment of memory disorders and the main active constituents may not be the known astragalosides (Tohda et al., 2006; Ghavami and Sardari, 2011). *A. mongholicus* is also used as an adjunctive therapy to chemo- and radiation therapy in cancer (Castegna et al., 2003). Recently, there are some reports on the neuroprotective activities of the crude extract from *A. mongholicus* (Arking et al., 2002).

The purpose of the present study was to demonstrate whether or not the neuroprotective property of *A. mongholicus* aqueous extract (AMAE) could have any effect on SCI in rats.
MATERIALS AND METHODS

Preparation of Astragalus mongholicus aqueous extract

The dried A. mongholicus were hammerd into small pieces and heated in 1000 ml distilled water for 5 h in water at 100°C. This process was repeated twice. The final yield of the aqueous extract used for this study was 47.6%.

Animals

Adult Wistar albino rats (190 to 220 g) of either sex were used for the pharmacological activities. They were kept in polypropylene cages at 25±2°C, with relative humidity 45 to 55% under 12 h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed (Poultry Research Station Tamilnadu Veterinary and Animal Sciences University, Chennai, India) and water ad libitum. The test extracts and the standard drugs were administered in the form of a suspension in water as suspending agent. Animals were divided into four groups: normal control, model control and two AMAE treatment groups.

Surgical procedure

After skin incision, dissection by planes was performed on the spinal process, detaching the spinotrapezium muscle from bone, and the vertebral layer was resected, exposing distal spinal cord meninges. Then, a cross-sectional transection of the spinal cord was performed with scissors. This procedure frequently caused meninges to bleed, being handled with compression of the affected portion with wet surgical gaueze. At last, muscle and skin layers were closed by planes with suture. All surgical procedure steps have been performed according to ethical procedures for the use of animals in laboratory experiments.

Biochemical analysis

Activation of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured by colorimetric method on an autoanalyser (Abbott, model Alcyon 300, USA) with RANSOD and RANSEL kits, respectively (RANDOX Laboratory, UK). Serum malondialdehyde (MDA) level was measured via reaction with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to generate a complex of pink color. Next, its fluorescence intensity was measured at 547 nm with excitation at 525 nm by a spectrophotometer (Kontron, model) (Del et al., 2003). GSH was measured by the method of Moron et al. (1979). Catalase (CAT) was measured by the method of Sinha (1972).

In situ terminal deoxynucleotidyl transferase assay (TUNEL method)

The TUNEL method is based on the specific binding of terminal deoxynucleotidyl transferase (TdT) to the 39-OH ends of DNA and the ensuing synthesis of a polydeoxynucleotide polymer. The TUNEL method was applied to 4% paraformaldehyde–fixed, paraffin-embedded sections 3 mm thick with an ApoTag in apoptosis detection kit (Oncor) according to the manufacturer’s instructions.

Histological studies and immunohistochemical staining

Specimen was fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. For immunohistochemical analysis, the enzymatic activity of endogenous peroxidases in the spinal cord section was first blocked with 3% hydrogen peroxide, followed by incubation with rabbit polyclonal antirat COX-2 antibody (Cayman Chemical CO., Ann Arbor, MI, USA) at room temperature for 40 min. The peroxidase binding sites were detected by staining with 3,3'-diaminobenzidine tetrahydrochloride (Dako, Glostrup, Denmark). Finally, counterstaining was performed using Mayer's hematoxylin.

Statistical analyses

Results were presented as mean ± standard deviation. Statistical analysis was carried out using students’ ‘t’ test to compare for significant difference between two means. Significant difference was considered at p<0.05 with the aid of Microsoft Excel 2003.

RESULTS AND DISCUSSION

Free radicals are well known reactive molecules mainly derived from univalent reduction of oxygen and giving rise to numerous by-products through reactions with almost all the unsaturated bonds found in natural living cells. The detrimental effects of such reactive molecules have been well described, since they can destabilize cell membranes by reacting with unsaturated fatty acids (Goto, 1982). Lipid oxidation products are well suited to induce arterial damage and based on their known cytotoxic effects, evidence also indicates the possibility of plaque formation and stimulation of thrombogenesis (Addis et al., 1995; Endres et al., 1995). LPO was monitored by measuring MDA resulting from free radical damage to membrane components of the cells.

Table 1 shows the levels of spinal cord MDA and GSH in rats. Spinal cord MDA level was higher in model rats, whereas GSH were lower than those in normal control rats. Supplementation with AMAE decreased the spinal cord MDA level and increased GSH level in AMAE-treated rats. This indicated that AMAE may reduce peroxidation lipid levels in AMAE-treated rats. Increased generation of reactive oxygen substance (ROS) and enhanced LPO are considered responsible for the toxicity of a wide range of compounds. Various authors have investigated relationships between AMAE and free radical reactions. Among the antioxidant enzymes, SOD catalyses dismutation of the superoxide anion (O2·−) into hydrogen peroxide, CAT detoxifies H2O2 and GSH-Px both detoxifies H2O2 and converts lipid hydroperoxides into non-toxic alcohols. These defenses provide protection from highly reactive free radical products which cause LPO and the destruction of biological molecules in the cell once the radicals are generated (Liochev and Fridovich, 1994; Levieux and Levieux, 1991; Shehata, 2012; Onyesom et al., 2011). Earlier studies reported increased LPO, increased LPO, increased or unaltered levels of GSH, decreased activity of GSH-Px and SOD in rats suffering from SCI.

Table 2 shows the levels of spinal cord SOD, CAT and GSH-Px in rats. Spinal cord SOD, CAT and GSH-Px activities were lower in model rats than those in normal rats. This indicated that AMAE may reduce LPO and the destruction of biological molecules in the cell once the radicals are generated (Liochev and Fridovich, 1994; Levieux and Levieux, 1991; Shehata, 2012; Onyesom et al., 2011). Earlier studies reported increased LPO, increased LPO, increased or unaltered levels of GSH, decreased activity of GSH-Px and SOD in rats suffering from SCI.

Table 3 shows the levels of spinal cord COX-2 in rats. Spinal cord COX-2 level was higher in model rats, whereas COX-2 were lower than those in normal control rats. Supplementation with AMAE decreased the spinal cord COX-2 level and increased COX-2 level in AMAE-treated rats. This indicated that AMAE may reduce COX-2 expression in rats suffering from SCI. Increased expression of COX-2 is responsible for the production of prostaglandin and other proinflammatory cytokines (Hoffmann et al., 2004; Endres et al., 1995). LPO was monitored by measuring MDA resulting from free radical damage to membrane components of the cells.
Table 1. Effect of AMAE on spinal cord MDA and GSH levels in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>4.31 ± 0.32</td>
<td>16.48 ± 1.85</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>7.84 ± 0.94**</td>
<td>9.57 ± 1.08**</td>
</tr>
<tr>
<td>A. mongholicus I</td>
<td>5.35 ± 0.72##</td>
<td>12.83 ± 1.37##</td>
</tr>
<tr>
<td>A. mongholicus II</td>
<td>4.02 ± 0.58##</td>
<td>15.12 ± 1.88##</td>
</tr>
</tbody>
</table>

** p<0.01, compared with normal control group; ## p<0.01, compared with SCI group.

Table 2. Effect of AMAE on spinal cord SOD, CAT and GSH-Px activities in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH-Px</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>231.4 ± 27.4</td>
<td>26.81 ± 1.74</td>
<td>38.29 ± 1.32</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>129.6 ± 15.2**</td>
<td>11.47 ± 2.05**</td>
<td>16.03 ± 1.44**</td>
</tr>
<tr>
<td>A. mongholicus I</td>
<td>188.4 ± 22.6##</td>
<td>19.57 ± 2.17##</td>
<td>24.66 ± 1.79##</td>
</tr>
<tr>
<td>A. mongholicus II</td>
<td>222.5 ± 26.9##</td>
<td>23.71 ± 2.59##</td>
<td>35.19 ± 2.61##</td>
</tr>
</tbody>
</table>

** p<0.01, compared with normal control group; ## p<0.01, compared with SCI group.

Table 3. Effect of AMAE on spinal cord apoptosis index in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Apoptosis index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.32 ± 0.32</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>16.94 ± 2.54**</td>
</tr>
<tr>
<td>A. mongholicus I</td>
<td>13.32 ± 3.52##</td>
</tr>
<tr>
<td>A. mongholicus II</td>
<td>10.73 ± 2.64##</td>
</tr>
</tbody>
</table>

** p<0.01, compared with normal control group; * p<0.05, ## p<0.01, compared with SCI group.

Table 4. Effect of AMAE on spinal cord iNOS positive expression rate in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Positive expression rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.06 ± 0.71</td>
</tr>
<tr>
<td>spinal cord injury</td>
<td>27.04 ± 4.36**</td>
</tr>
<tr>
<td>A. mongholicus I</td>
<td>20.11 ± 4.84##</td>
</tr>
<tr>
<td>A. mongholicus II</td>
<td>13.28 ± 6.09##</td>
</tr>
</tbody>
</table>

** p<0.01, compared with normal control group; ## p<0.01, compared with SCI group.

Control rats. Supplementation with AMAE increased the spinal cord SOD, CAT and GSH-Px activities in AMAE-treated rats. We recorded increased LPO, decreased GSH levels, decreased GSH-Px, CAT and SOD activities in the spinal cord of rats. Our study revealed that AMAE can enhance antioxidant enzymes activities in the spinal cord of AMAE-treated rats.

SCI results in locomotor dysfunction below the level of injury, partly due to interruption of descending pathways that transmit motor commands from the brain to the spinal cord (Kim et al., 2003). As mentioned previously, primary injury can lead to secondary injury which often involves apoptosis. Apoptosis is an active gene-directed cell death process that plays an important role in the development of many diseases (Casha et al., 2005).

A significant increase in the spinal cord apoptosis index (Table 3) of the rats in model group was found (p<0.01). As can be seen, after the trial the spinal cord apoptosis index in the groups of rats fed AMAE had decreased significantly (p<0.05, p<0.01).

Nitric oxide (NO) is a gaseous free radical generated in most cells as a result of a diverse range of stimuli. This molecule may show protective effects in the nervous system, although pathologically elevated levels result in cytotoxicity. There are three major forms of enzyme that synthesize NO from L-arginine: the so-called NO synthases (NOS), with a 50 to 60% sequence homology between species (Lamas et al., 1992; Mohany et al., 2011); neuronal (nNOS or NOS I) and endothelial (eNOS or NOS III) types, comprising the constitutive isoforms; and finally the inducible type (iNOS or NOS II). A fourth subtype of NOS (mtNOS) is an isoform of nNOS, and has been found in the inner mitochondrial membrane of several tissues including those of the liver, brain, heart and muscles (Ellerling et al., 2002; Navarro and Boveris, 2008; Saeed and Musallam, 2011).

The effect of AMAE on the spinal cord iNOS positive expression rate in rats after given for a period of 12 days is shown in Table 4. Spinal cord iNOS positive expression rate was found to be significantly increased in model animals. Administration of AMAE significantly dose-dependently decreased spinal cord iNOS positive expression rate in AMAE rats.

Conclusion

In short, increased oxidative injury, apoptosis index and
iNOS positive expression rate is detected in spinal cord of rats suffering from SCI. Administration of AMAE can decrease oxidative injury, apoptosis index and iNOS positive expression rate is detected in spinal cord of rats suffering from SCI.

REFERENCES


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REFERENCES


