Effect of coenzyme Q₁₀ on spinal cord ischemia-reperfusion injury

Jin-Young Hwang, MD, PhD,† Seong-Won Min, MD, PhD,† Young-Tae Jeon, MD, PhD,† Jung-Won Hwang, MD, PhD,‡ Sang-Heon Park, MD, PhD,‡ Jin-Hee Kim, MD, PhD,‡ and Sung-Hee Han, MD, PhD‡

†Department of Anesthesiology and Pain Medicine, SMG-SNU Boramae Medical Center; and ‡Department of Anesthesiology and Pain Medicine, Seoul National University Bundang Hospital, Gyeonggi-do, Republic of Korea

OBJECT Spinal cord ischemia remains a serious complication of thoracoabdominal aortic aneurysm surgery. Coenzyme Q₁₀, a potent antioxidant, has been reported to exert a neuroprotective effect. In the present study, we evaluated the effect of coenzyme Q₁₀ pretreatment on spinal cord ischemia-reperfusion injury.

METHODS Male Sprague-Dawley rats were treated with either 300 mg/kg coenzyme Q₁₀ (CoQ₁₀ group, n = 12) or saline (control and sham groups, n = 12 for each group) for 5 days before ischemia. Spinal cord ischemia was induced in the control and CoQ₁₀ groups. Neurological function was assessed using the Basso-Beattie-Bresnahan (BBB) motor rating scale until 7 days after reperfusion, and then the spinal cord was harvested for histopathological examinations and an evaluation of malondialdehyde level.

RESULTS On post-reperfusion Day 1, the CoQ₁₀ group showed higher BBB scores compared with those in the control group, although the difference was not significant. However, on Day 2, the CoQ₁₀ group showed a significantly higher BBB score than the control group (14.0 [10.3–15.0] vs 8.0 [5.0–9.8], median [IQR], respectively; p = 0.021), and this trend was maintained until Day 7 (17.5 [16.0–18.0] vs 9.0 [6.5–12.8], respectively; p < 0.001). Compared with the control group, the CoQ₁₀ group had more normal motor neurons (p = 0.003), fewer apoptotic changes (p = 0.003) and a lower level of tissue malondialdehyde (p = 0.024).

CONCLUSIONS Pretreatment with 300 mg/kg coenzyme Q₁₀ resulted in significantly improved neurological function and preservation of more normal motor neurons.


KEY WORDS coenzyme Q₁₀; spinal cord ischemia; thoracoabdominal aortic aneurysm surgery; spinal cord injury

PARAPARESIS or paraplegia is a serious complication following thoracoabdominal aortic aneurysm surgery and is a consequence of spinal cord ischemia-reperfusion injury secondary to aortic clamping and declamping. A series of processes, including the glutamate-mediated excitotoxicity, inflammation, and apoptosis involved in spinal cord ischemia-reperfusion and oxidative stress, have emerged as key mechanisms of neuronal ischemia-reperfusion injury. Ischemia-induced malfunction of the oxidative respiratory chain in mitochondria results in a burst of reactive oxygen species (ROS), and the ROS generated from mitochondria damages ischemic neurons by direct attack on cellular constituents such as lipids and nucleic acids and by acting as a molecular trigger for apoptosis and necrotic cell death.

Coenzyme Q₁₀ (CoQ₁₀) is an endogenous provitamin present primarily in the mitochondria and plays an important role in the electron transport chain. It participates in aerobic cellular respiration, generating energy in the form of adenosine triphosphate, and serves as a potent antioxidant in lipid membranes and mitochondria.

Exogenous CoQ₁₀ has been widely applied to dietary supplements, and could be suggested as a therapeutic agent for cardiovascular diseases. CoQ₁₀ also possesses neuroprotective properties. Administration of exogenous CoQ₁₀ offers neuroprotective effects in various models of central
nervous system disorders, including ischemic stroke.\textsuperscript{13,17} The related mechanisms of the neuroprotective effect of CoQ\textsubscript{10} are modulation of oxidative stress and attenuation of apoptotic cell death.\textsuperscript{20}

The important role of oxidative stress in spinal cord ischemia-reperfusion injury suggests that CoQ\textsubscript{10} may have a protective effect,\textsuperscript{22} but the effect of CoQ\textsubscript{10} on spinal cord ischemia has not been determined. We hypothesized that CoQ\textsubscript{10} may improve neurological outcome following spinal cord ischemia-reperfusion injury and evaluated its effect in a rodent model in terms of motor function, histopathological findings, and level of oxidative stress.

Methods

This study was approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital. Animal experiments and care were conducted in accordance with the \textit{Guide for the Care and Use of Laboratory Animals}.

All the animals were kept at room temperature with equal lighting control (12-hour light/12-hour dark cycle). All the surgical procedures and post-reperfusion neurological assessment were started at 10 AM.

Experimental Groups and Surgical Preparation

Male Sprague-Dawley rats (300–350 g, n = 36) were randomly assigned to one of 3 groups: 1) sham group (n = 12), which received orally administered saline daily for 5 days before surgery; 2) control group (n = 12), which also received orally administered saline daily for 5 days before surgery; or 3) coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) group (n = 12), which received 300 mg/kg of orally administered CoQ\textsubscript{10} daily for 5 days before induction of ischemia. Oral administration was performed via oral gavage using a 16-gauge feeding needle.

Rats were anesthetized in an acrylic box with isoflurane (5 vol\%) in 100% oxygen. After induction, anesthesia was maintained with a facial mask of inhaled 1.0–2.5 vol\% isoflurane driven by oxygen flow at 2 L/minute. Rats were placed in the supine position and the hair in the left inguinal area and neck was shaved. The tail artery was exposed and cannulated with a polyethylene catheter (PE-50) for injection of heparin and monitoring of distal arterial pressure. The left femoral artery was exposed to induce spinal cord ischemia, and a 2-Fr Fogarty catheter (Fogarty arterial embolectomy catheter, Edwards Lifesciences) was inserted into the descending thoracic aorta so that the tip of the catheter reached the left subclavian artery (11 cm from the insertion site).\textsuperscript{21} The left carotid artery was exposed and cannulated with a 20-gauge catheter (BD Insyte, Becton Dickinson) to control the proximal arterial pressure. The rats’ body temperature was monitored with a rectal probe and maintained at 37.5°C ± 0.5°C with a heating blanket and overhead lamp.

Spinal Cord Ischemia

Spinal cord ischemia was induced by investigators who were blinded to the group assignments (Fig. 1). After completion of cannulation, heparin (150 U) was injected into the tail artery and the balloon of the Fogarty catheter was inflated with 0.05 ml of saline. The blood was simultaneously drained from the carotid artery into the external reservoir filled with heparinized saline to maintain the proximal arterial pressure at 80 mm Hg during aortic occlusion. The success of aortic occlusion was confirmed by an immediate drop and sustained loss of distal arterial pressure. Animals in the sham group were prepared in the same manner, but spinal cord ischemia was not induced. After 10 minutes and 30 seconds of aortic occlusion, the Fogarty balloon was deflated, and the drained blood was reinfused. After the removal of all catheters and the wound closure, the rats recovered from anesthesia.

Evaluation of Physiological Parameters

Mean arterial pressure, heart rate and body temperature were monitored continuously. The values were recorded 10 minutes before aortic occlusion, 5 minutes after aortic occlusion and 10 minutes after reperfusion. Arterial blood gas and hematocrit levels were measured 10 minutes before aortic occlusion and 10 minutes after reperfusion.

Hind Limb Motor Function

Motor function was examined at 1, 2, 3, 5, and 7 days after reperfusion by 2 observers who were blinded to the group assignments. Hind limb motor function was assessed using the Basso-Beattie-Bresnahan (BBB) motor rating scale.\textsuperscript{2} The scale has 22 levels, which range from 0 (no observed hind limb movement) to 21 (normal locomotion). Values of 1–8 are scored for small or large movements of the 3 hind limb joints, a score of 9 is given for attainment of plantar weight bearing or dorsal stepping, and scores of 10–20 involve progressive improvements in coordinated walking ability. After the last neurological assessment, rats in each group were divided into 2 subgroups for histopathological examination (n = 7 per group) or oxidative stress evaluation (n = 5 per group).
Histopathological Evaluation

Seven rats per group were deeply anesthetized with isoflurane and were perfused transcardially with 100 ml of heparinized saline. The spinal cords were removed and fixed in 10% buffered formalin for 24 hours, and spinal cord segments from the L3–5 level were embedded in paraffin. Transverse sections were cut at a thickness of 4 µm and stained with hematoxylin and eosin (H & E). Neurodegenerative injury was evaluated at a magnification of 200 by an investigator blinded to the group assignments. To assess the degree of ischemic neuronal injury, the number of non-necrotic motor neurons in the anterior horn of the spinal cord (anterior to a line drawn through the central canal perpendicular to the vertebral axis) was counted in 3 sections for each animal and averaged.24 Terminal deoxynucleotidyl transferase (TdT)–mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining was performed following the protocol provided by the manufacturer (S7100, Millipore). Spinal cord sections were deparaffinized, rehydrated, digested with 20 µg/ml proteinase K (Sigma Chemical) for 30 minutes at room temperature, quenched with 3% hydrogen peroxide for 5 minutes at room temperature, rinsed in phosphate-buffered saline (PBS), and subsequently incubated with a reaction solution composed of 55 µl/5 cm² of working strength TdT enzyme under a humidified atmosphere for 60 minutes at 37°C. After rinsing in PBS, the sections were incubated for 30 minutes at room temperature with anti-digoxigenin antibody. Immunoreaction products on sections were developed at 434°C for 60 minutes and centrifuged. The absorbance at 586 nm was then recorded.

Oxidative Stress Evaluation

Five rats per group were deeply anesthetized, and then their spinal cords were extracted after transcardial perfusion of ice-cold saline. To evaluate oxidative stress levels, the tissue malondialdehyde (MDA) was measured. MDA is the end product of lipid peroxidation and is widely used as an indicator of oxidative stress.7 The tissue MDA level was measured according to the manufacturer’s protocol (MDA-586TM, OxisResearch, Percipio Biosciences). Briefly, the spinal cord was homogenized in PBS (1:10, w:v). The homogenate was centrifuged, and aliquots of the supernatant were treated with N-methyl-2-phenylindole at 45°C for 60 minutes and centrifuged. The absorbance at 586 nm was then recorded.

Statistical Analysis

The SPSS software version 20 was used for statistical analysis (IBM Inc.). Data are expressed as medians and interquartile ranges (IQRs). Numerical data were compared using the Kruskal-Wallis test, followed by the post hoc Mann-Whitney U-test. The Bonferroni-adjusted p value was obtained by multiplying the unadjusted p value by the number of comparisons (i.e., 3). A repeated-measures ANOVA was used to evaluate the differences in locomotor function over time between the control and CoQ10 groups. A p value < 0.05 was considered to indicate significance.

Results

The mean distal arterial pressure, heart rate, and rectal temperature were similar among the 3 groups at all time points (Table 1). No significant between-groups differences were observed for arterial pH, PaO₂, or PaCO₂ at any time point. Hematocrit values before aortic occlusion were similar among the 3 groups. After reperfusion, both the control and CoQ10 groups showed significantly decreased hematocrit values compared with those in the sham group (p = 0.003), but no difference was observed between the control and CoQ10 groups (Table 2).

All animals survived until the final neurological as-
assessment at 7 days after the surgical procedure. The sham group had a BBB score of 21 throughout the experimental period. On postoperative Day 1, the CoQ10 group tended to show higher BBB scores compared with the control group, although the difference was not significant. However, the BBB score was significantly higher in the CoQ10 group than in the control group on postoperative Days 2, 3, 5 and 7 (Fig. 2). The BBB score changed significantly over time in the control and CoQ10 groups (p < 0.001, respectively) and a significant difference was observed in the change in BBB score over time between the two groups (p < 0.001).

The number of normal motor neurons in the anterior spinal cord is presented in Fig. 3. The CoQ10 group had significantly fewer normal motor neurons compared with the sham group. However, the CoQ10 group displayed a higher number of normal motor neurons compared with the control group. The CoQ10 group showed significantly fewer apoptotic nuclei (expressed as a percentage of the total number of nuclei) than the control group (median 36.6% [IQR 33.5%–40.0%] vs 51.8% [46.7%–60.5%]; p = 0.003). Representative photographs of spinal cord sections stained with H & E are shown in Fig. 4.

### TABLE 2. Hematological parameters*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n = 12)</th>
<th>Control (n = 12)</th>
<th>CoQ10 (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before occlusion</td>
<td>7.40 (7.38–7.41)</td>
<td>7.40 (7.39–7.41)</td>
<td>7.41 (7.38–7.41)</td>
</tr>
<tr>
<td>After reperfusion</td>
<td>7.38 (7.36–7.40)</td>
<td>7.37 (7.34–7.38)</td>
<td>7.37 (7.36–7.39)</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before occlusion</td>
<td>231.6 (204.7–275.7)</td>
<td>226.7 (203.7–275.7)</td>
<td>220.0 (211.3–231.2)</td>
</tr>
<tr>
<td>After reperfusion</td>
<td>249.3 (225.4–282.7)</td>
<td>250.5 (226.6–282.7)</td>
<td>249.3 (200.3–260.6)</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before occlusion</td>
<td>36.5 (35.2–36.9)</td>
<td>35.9 (34.8–37.4)</td>
<td>35.3 (33.9–37.0)</td>
</tr>
<tr>
<td>After reperfusion</td>
<td>35.8 (34.6–36.4)</td>
<td>36.4 (34.5–38.3)</td>
<td>36.0 (34.8–38.3)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before occlusion</td>
<td>38.5 (36.0–41.3)</td>
<td>39.5 (36.0–41.3)</td>
<td>40.0 (38.0–41.3)</td>
</tr>
<tr>
<td>After reperfusion</td>
<td>39.0 (37.8–41.0)</td>
<td>36.5 (34.8–38.0)</td>
<td>36.0 (35.0–37.3)</td>
</tr>
</tbody>
</table>

* Values are presented as medians (interquartile range). No differences in blood gas data were found at any time point. Hematocrit did not differ between the 3 groups before aortic occlusion, but both the control and CoQ10 groups showed significantly lower hematocrit values after reperfusion compared to those in the sham group.

† p < 0.05 compared to the sham group.

---

**FIG. 2.** Scores on the BBB locomotor rating scale. Values are presented as medians (interquartile range). Animals in the sham group and control group received saline orally for 5 days before surgery; animals in the CoQ10 group received 300 mg/kg coenzyme Q₁₀ orally for 5 days before surgery. Aortic occlusion was performed in the control and CoQ10 groups. *p < 0.05 compared with the sham group. †p < 0.05 compared with the control group.

**FIG. 3.** Comparison of numbers of normal motor neurons in the anterior horn of the spinal cord in specimens from the 3 groups. The number of normal neurons per section was counted in 3 sections per animal and an average number per section was calculated for each animal. The bars indicate the median of these averaged values for each group, and error bars indicate the IQR. *p = 0.003 between the control and CoQ₁₀ groups. †p = 0.021 between the CoQ₁₀ and sham groups.
Figure 5 presents spinal cord tissue MDA levels. The CoQ10 group exhibited a significantly lower level of tissue MDA compared with the control group (p = 0.024). No significant difference was observed in the tissue MDA level between the CoQ10 and sham groups.

Discussion
We showed that pretreatment with CoQ10 significantly improved motor function in a rat model of spinal cord ischemia-reperfusion injury. The reduced oxidative stress and attenuated neuronal apoptosis could be part of the mechanisms of CoQ10’s protective effect. This is the first study to show the neuroprotective effect of CoQ10 against spinal cord ischemia-reperfusion injury.

CoQ10 has been widely postulated to have neuroprotective properties, and administration of exogenous CoQ10 has been shown to exert neuroprotective effects in various models of CNS disorders, including neurodegenerative disease,13,25 epilepsy,1 and ischemic injury.17,18,26 In a preliminary clinical trial, CoQ10 treatment after cardiac arrest improved survival rate and neurological outcomes in survivors when combined with mild hypothermia.5 More recently, it was found that CoQ10 is protective against diabetic neuropathy in animal diabetes models.19 Our finding that the groups treated with CoQ10 showed significantly improved motor function was consistent with these reports.

In the present study, MDA content in the spinal cord tissue was significantly lower in rats receiving CoQ10 compared with that in the control group. MDA is the end product of lipid peroxidation, and has been used as an indicator of oxidative stress in spinal cord injury models.6,23 Our findings, which demonstrated the decreased oxidative stress following CoQ10 administration, agree with previous studies that used various cerebral injury models.1,10,25 In an experimental traumatic brain injury model, the group treated with CoQ10 after head trauma showed significantly lower MDA levels and attenuated neuronal damage compared with the control group.19
of temporal lobe epilepsy, CoQ_{10} pretreatment effectively reduced the increase of kainate-induced intrahippocampal MDA level and attenuated hippocampal neuronal loss.\textsuperscript{1} CoQ_{10} supplementation also decreased the MDA level and attenuated beta-amyloid pathology in an Alzheimer disease model.\textsuperscript{23} In addition, previous studies using a cerebral ischemia model indicate that CoQ_{10} exerts a neuroprotective effect by attenuating the severity of oxidative stress.\textsuperscript{16,17} In a rat model of cerebral ischemia induced by endotoxins, CoQ_{10} effectively diminished neuronal injury by decreasing free radical concentration.\textsuperscript{16} Our findings that animals treated with CoQ_{10} showed a reduced level of spinal cord MDA contents and improved neurological deficit are consistent with these previous reports and suggest that the neuroprotective effect of CoQ_{10} against spinal cord ischemia-reperfusion injury is related to its antioxidative activity.

Under oxidative stress, neuronal cells undergo a series of processes related to cellular necrosis or apoptosis,\textsuperscript{3} and the ROS generated from mitochondria are a key mediator of molecular signaling for neuronal apoptosis and cell death.\textsuperscript{19} Our histopathological findings from the spinal cord sections at Day 7 showed more preserved normal motor neurons and fewer cellular apoptotic changes in rats receiving CoQ_{10} compared with the control group, in accordance with earlier studies.\textsuperscript{4,11,26} In an animal model of cerebral ischemia, CoQ_{10} intake reduces programmed cell death following hippocampal ischemia-reperfusion injury, which was mediated by the inhibition of neuronal apoptotic pathway by increasing Bcl2 expression, an antiapoptotic protein and suppressing Bax, a pro-apoptotic protein.\textsuperscript{26} In an in vitro model of cortical neuronal injury, CoQ_{10} effectively protects neuronal cells against amyloid beta-induced apoptosis by inhibiting oxidative stress and activating cellular surviving signals.\textsuperscript{4} In addition, CoQ_{10} exerts a neuroprotective effect against oxidative stress-induced apoptosis in dopaminergic neurons.\textsuperscript{11} It should be noted that we observed that pretreatment with CoQ_{10} reduced motor neuronal apoptosis and oxidative stress, but there might be other processes that have not been assessed in our study. CoQ_{10} may modulate several cellular processes associated with the neuroprotective effect against spinal cord ischemia-reperfusion injury. Further studies are required to determine the mechanisms of the neuroprotective effect of CoQ_{10}.

Conclusions

Pretreatment with 300 mg/kg CoQ_{10} orally for 5 days before ischemia significantly improved neurological outcomes at 2, 3, 5 and 7 days and prevented motor neuronal apoptosis after spinal cord ischemia in rats.

References


Author Contributions
Conception and design: Han, JY Hwang, Min, Jeon, JW Hwang, Kim. Acquisition of data: Han, JY Hwang, Min, Jeon, JW Hwang, Park. Analysis and interpretation of data: Han, JY Hwang. Drafting the article: Han, JY Hwang. Approved the final version of the manuscript on behalf of all authors: Han.

Correspondence
Sung-Hee Han, Department of Anesthesiology and Pain Medicine, Seoul National University Bundang Hospital, 166 Gumi-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, Republic of Korea. email: anesthesiology@snubh.org.