Curcumin Inhibits Apoptosis and Brain Edema Induced by Hypoxia-Hypercapnia Brain Damage in Rat Models

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Abstract: Curcumin, extracted from South Asian spice turmeric, has been determined to have the promising ability in antioxidation and anti-inflammation. However, the effect of curcumin on treating brain damage has been not reported. In this article, the aim was to evaluate the effect of curcumin on cell apoptosis in rats exposed to hypoxia-hypercapnia and explore the therapeutic potential of curcumin in hypoxia-hypercapnia brain damage (HHBD). Sprague Dawley rats were randomly assigned into 3 groups: control group, hypoxia-hypercapnia group and curcumin group. The Fas/FasL expressions in HHBD rats treated by curcumin were measured by immunohistochemical staining and western blotting. The pathological changes of brain cells were observed by transmission electron microscope. Rats with HHBD showed significant increase of Fas/FasL expression and ultrastructural changes in brain tissue cells. Curcumin intervention effectively reversed the Fas/FasL-mediated apoptosis and HHBD-induced brain edema. Curcumin may be a potential therapeutic alternative for HHBD.

Key Indexing Terms: Cell apoptosis; Fas/FasL expression; Ultrastructure changes. **[Am J Med Sci 2015;349(6):521–525.]**

Hypoxia-hypercapnia brain damage (HHBD) is one of the main complications of respiratory failure.[1] The main characteristics of brain damage are associated with brain edema, neuronal apoptosis and acidosis.[2] Acidosis induced by hypercapnia extends the injury of hypoxia-ischemic brain damage.[3] HHBD can result in nervous system disorders, mental retardation and even death. The disability rate and mortality resulting from HHBD are high among neonates and adults. HHBD is a potential serious threat to public health.

Great progresses have been achieved in exploring the effective therapy for preventing brain damage. Curcumin, served as a diarylheptanoid, is the main compound of popular South Asian spice turmeric belonging to ginger family. Curcumin has been used as a natural food additive for the color stability and low toxicity[4] and proven to be effective in antioxidation and anti-inflammation.[5,6] Recent evidences indicate that curcumin can relieve the hypoxic-ischemic brain injury by suppressing the activities of nitric oxide synthase and expression of water channel protein 4 (Aquaporin-4, AQP-4).[7] Curcumin may be a candidate agent for brain damage prevention, but the evidences seemed to be insufficient.

Cell apoptosis is determined to be an event in the pathophysiologic mechanism of brain damage.[8] The Fas and its ligand (FasL) play key roles in regulating apoptotic cell death and neurologic disorders induced by brain injury.[9] In this study, the authors evaluated the expression of Fas/FasL protein and morphological changes in brain cells. The purpose of this study was to explore the effect of curcumin on cell apoptosis induced by HHBD and provide a candidate therapy for preventing brain injury induced by hypoxia and hypercapnia.

MATERIALS AND METHODS

Approval was obtained from the Ethics Committee of Wenzhou Medical University, and all the animal studies were performed according to the ethical standards.

Animals

Specific pathogen-free Sprague Dawley rats were provided by experimental animal center of Wenzhou Medical University. Two-month-old male Sprague Dawley rats weighting from 180 to 220 g (mean = 200 ± 20 g) were used in this study.

Groups and Hypoxia-Hypercapnia Exposure

Animals were kept in a 40% to 70% humidity environment at 22 to 25°C and had free access to food and water for 1 week. Then, the rats were assigned randomly into 3 groups: (1) control group (n = 6), (2) hypoxia-hypercapnia group (n = 6) and (3) curcumin group (n = 6).

Animals in control group were maintained at normobaric conditions (760 mm Hg). Rats in hypoxia-hypercapnia and curcumin groups were exposed to gas mixtures containing 8% to 11% O_2 and 3.0% to 5.0% CO_2 in a specially fabricated animal normobaric chamber. Animals were treated with gas mixture for 8 hours daily, 6 days a week for consecutive 4 weeks. Additionally, rats in curcumin group were treated with curcumin suspension (Sigma, Louis, MO; 150 $mg \cdot kg^{-1} \cdot d^{-1}$) intragastricly, 1 hour before exposed to the hypoxia-hypercapnia environment. At the end of a consecutive 4-week period, all the animals were euthanized by hemospasia. The brain tissues of the rats were removed and stored at −80°C for further analysis.

Immunohistochemistry

The immunohistochemistrical staining was performed to evaluate the Fas and FasL expression in brain cells based on the method described previously.[10] The tissue of cerebral cortex (1.5 × 1.5 × 0.3 cm) was fixed in 4% paraformaldehyde for 2 hours at room temperature and routinely embedded in paraffin. Serial sections (4 μm) were mounted on glass slides and processed for immunohistochemistrical staining. The sections were deparaffinized and treated with 3% H_2O_2 methanol solution, followed by blocking with 2% goat serum in Tris-buffered saline. The antibodies were diluted in Tris Buffered Solution +Tween

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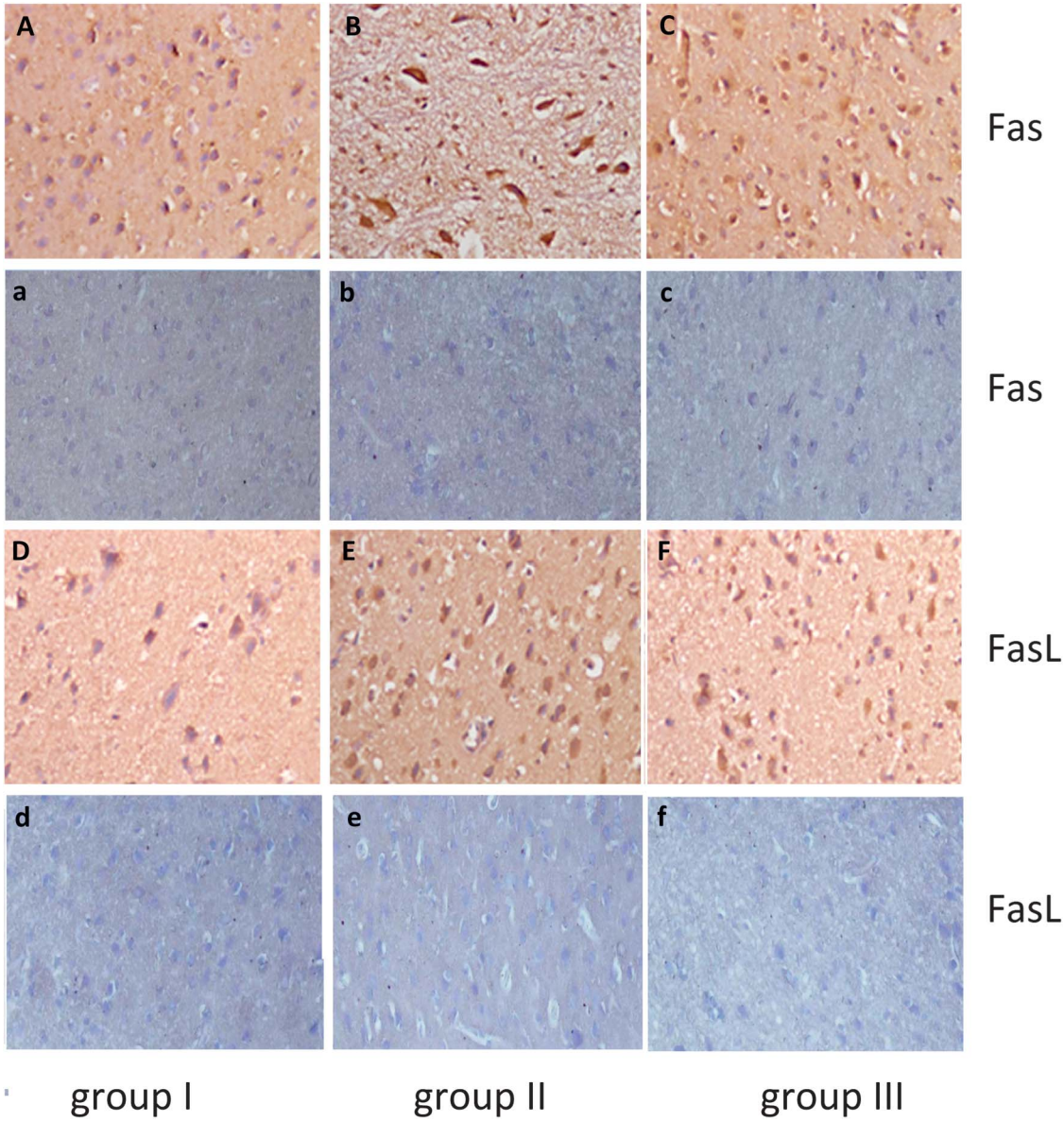

FIGURE 1. Immunohistochemical staining for Fas/FasL positive cells of the brain tissue in different groups (magnification, 400×). (A–C) Membranes were incubated with the primary antibody of rabbit antibodies against Fas and the 2nd antibody of horseradish peroxidase (HRP)-conjugated goat antibodies against rabbit. (D–F) The cells were treated with rabbit antibodies against FasL primary antibody and HRP-conjugated goat antibodies against rabbit. (a–f) The cells were treated with the 2nd antibody only. Compared with controls, there is no specific staining in samples.

solution (150 mmol/L NaCl, 50 mmol/L Tris-HCl, pH 7.4 and 0.05% Tween 20). Then, the sections were incubated with rabbit antibodies against Fas (1:800; Santa Cruz, California) or FasL (1:800; Santa Cruz) as the primary antibodies and horseradish peroxidase–conjugated goat antibodies against rabbit as the 2nd antibody. After the sections were stained with 3, 3′ diaminobenzidine method, the Fas/FasL positive cells were observed under microscope. The percentage of Fas/FasL positive cells to total brain cells was calculated by image analysis of 5 random fields (magnification, 400×).

Western Blot Analysis

After unfrozen, a patch of cerebral cortex tissues were cut for protein extraction. The brain tissues were homogenized in ice-cold lysate buffer containing 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate, 0.1% protease inhibitor and phosphatase inhibitor. The proteins were isolated by centrifugalization at 12,000 rpm for 5 minute. The protein content was determined by bicinchoninic acid assay. Proteins samples (25 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis system and transferred to nitrocellulose membranes. Membranes were blocked in 5% nonfat milk for 1 hour at room temperature and incubated with primary rabbit antimouse FaS/FaSL antibody (1:500 dilution; Santa Cruz) or anti-GAPDH (antiglyceraldehyde-3-phosphate dehydrogenase) antibodies (loading control; 1:1000; Santa Cruz) overnight at 4°C. Then, the membranes were incubated with horseradish peroxidase–conjugated goat antirabbit antibody (1:500) for