Effect of Early Intervention with Extract of Huannao Yicong Decoction (还脑益聪方) on the Pathologic Picture of Hippocampus and Neurocyte Apoptosis in APP Transgenic Mice Model of Dementia

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ABSTRACT  Objective: To observe the effect of early intervention using extract of Huannao Yicong Decoction (还脑益聪方, HYD) on the pathological picture of hippocampus, neurocyte apoptosis, and associated regulatory genes in β-amyloid precursor protein (APP) transgenic mice model of dementia. Methods: Sixty APP695V7171 transgenic mice were randomly divided into four groups of 15. The model group was treated with distilled water, the positive control group was treated with donepezil (0.65 mg/kg), and the two HYD groups were treated with high dose (2.8 g/kg) and low dose (1.4 g/kg) HYD, respectively. All testing drugs were administered through gastrogavage by dissolving in equal volume of distilled water, once a day for six successive months. In addition, a normal control group with 15 healthy C57BL/6J mice of the same age and genetic background was set up with distilled water treatment. The pathologic picture of brain tissue was observed by microscopy with HE stain; the amount of apoptosis cells in the hippocampal CA1 area was detected by TUNEL; and expressions of associated genes, Bcl-2, and Bax were determined by immunohistochemical method. Results: Pathologic pictures of hippocampus showed that in the model group, cells messily arranged, neurons markedly decreased, and the surrounding tissue of some cells was loosened with edema, necrosis, and widened gap with glia cells proliferation. Compared with those in the normal group, the amount of apoptosis cells in the CA1 area was increased, Bcl-2 expression decreased, and Bax expression increased significantly, with markedly reduced Bcl-2/Bax ratio in the model group. Compared to the model group, the pathological changes were significantly milder in the HYD-treated groups, showing rather regularly arranged cells, significantly increased neurons, only few denatured necrotic cells with milder edema, less proliferation of glia cells, and obviously reduced cell apoptosis in CA1 area (P<0.05 or P<0.01). Besides, Bcl-2 expression was up-regulated and Bax expression down-regulated, and Bcl-2/Bax ratio significantly increased in the two HYD groups (P<0.05 or P<0.01). Conclusion: Early intervention with HYD could improve the abnormal pathologic picture of hippocampus and regulate the expressions of associated genes to suppress cell apoptosis, which might be its mechanism of action in alleviating cognitive functional disorder.

KEYWORDS  β-amyloid precursor protein transgenic mice, extract of Huannao Yicong Decoction, cell apoptosis

Alzheimer's disease (AD) is a central nerve degenerative disease that manifests chiefly symptoms of learning/memory dysfunction. In addition to the typical features of neurofibril tangle and senile plaque, the pathological changes found in AD are deletions of synapse and neurons in cortex and hippocampus, in which the change in the hippocampus occurs in advance to that in cortex. Apparent deficit of neurons could be found in the hippocampal CA1 area at an early stage, which is considered to be related with cell apoptosis\(^\text{1,2}\).

Previous studies conducted by the authors have proved that Huannao Yicong Decoction (还脑益聪方, HYD) demonstrated a positive effect in treating senile
cognitive function disturbance in clinical practice\(^{(3,4)}\) and favorable regulation on hippocampal neuron apoptosis and its associated regulatory genes in experimental dementia model rats\(^{(5)}\). In order to further explore its mechanism of action in improving learning/memory function, HYD was used for early intervention on a three-month old transgenic mice model of dementia made by \(\beta\) -amyloid precursor protein (APP) to observe its effects on pathological picture, neuron apoptosis, and regulation of associated genes in mice's hippocampus. The study is reported as follows.

**METHODS**

**Experimental Animal**

Sixty APP695\(^{V717I}\) transgenic mice, three months old, half of which were male, the other half were female, weighing 17–23 g, and 15 C57BL/6J mice of same age and body weight, with same genetic background were purchased from the Experimental Animal Institute of Chinese Academy of Medical Sciences, certification No. SCXK (Jing) 2005-0013, which were bred in a room of clean grade.

**Testing Drugs**

HYD, consisting of *Radix Polygoni Multiflori*, *Radix Ginseng*, *Rhizoma Ligusticum wallichii*, *Rhizoma Acorus Graminei*, and *Rhizoma Coptis*, with a ratio of 2.4:2:1.8:1.2:1 was prepared by the National Key Laboratory for Available Utilization of Chemical Engineering Resource, Beijing Chemical Engineering University with the crude drugs provided by Shennong Co., Ltd., of Chinese Medicinal Herbs, Anguo City, Hebei Province. The extraction was made in the optimal condition chosen by orthogonal design. The contents of the three composites in the extract, diphenyl-vinylaminine, ferulic acid, and berberine hydrochloride were determined by HPLC as 3.58%, 0.32%, and 1.83%, respectively; the active composite transferring rates of all five crude drugs were >50%. The contents of various ingredients in the final prepared extract, determined by ultraviolet visible spectrophotometer (colorimetric), were composed of total flavone 14.19%, total saponin 25.98%, total phenolic acid 5.2%, and polysaccharide (precipitated) 51.36%. Donepezil (DP), 5 mg/tablet, was provided by the Weicai Pharmaceutical Co., Ltd., China, Batch No. 081121A.

**Reagents and Instrument**

Peroxidase-labeled streptavidin immuno-histochemical staining kit, Batch No.100113, and 3,3 diaminobenzidine (DAB) staining kit, Batch No.100119, were provided by Bioss Biotechnic Co., Ltd.; TdT-mediated dUTP nick end labeling (TUNEL) cell apoptosis test kit was provided by Beijing Zhongshan Jinqiao Biotechnic Co., Ltd., Batch No. 20091209; rabbit Bax antibody (1:100), Batch No. 20100116 and rabbit Bcl-2 antibody (1:200), Batch No. 20100122, were products of Wuhan Boster Biotechnic Co., Ltd., purchased from Beijing Baishi Chuangxin Scientific Technology Co., Ltd.; biomicroscope type BH-2 was a product of Olympus Co., Japan; Dpx View Pro computerized color image processor was a product of Delata Pix Co., Denmark.

**Animal Grouping and Treatment**

APP695\(^{V717I}\) transgenic mice were randomly allocated into four groups, 15 for each group, e.g., the model group was treated with distilled water; the positive control group was treated with 0.65 mg/kg (equivalent to the dosage used in human adult in clinic) of DP; and the two HYD groups were treated with high dosage (2.8 g/kg, two-fold of dosage used in human adult in clinics) and low dosage (1.4 g/kg, equivalent to the dosage of human adult in clinics), respectively. The testing drugs were dissolved in equal volume distilled water administered through gastrogavage, once a day for six successive months. Also, a normal control group set up with 15 C57BL/6J mice of the same age with the same genetic background was infused with equal volume distilled water by gastrogavage.

**Brain Sampling Preparation**

After the mice were sacrificed by decapitation, their brains were rapidly taken out under low temperature, dried with filter paper, and weighted. Brains of six mice selected randomly from each group were fixed in 4% paraform solution for over 24 h and then changed the fixing solution. The frozen section was carried out after the brain tissue subsided down, and the slice was paraffin embedded and stained.

**Pathologic Picture of Hippocampus**

Routinely sectioned 4–5 \(\mu\) m slice of brain tissue was HE stained, dehydrated, transparentized, and mounted, and then, the pathologic picture was observed under a light microscope.