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Protective effect of gypenosides on DNA and RNA of rat neurons in cerebral ischemia-reperfusion injury

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KEY WORDS gypenosides; brain ischemia; reperfusion injury; hippocampus; cerebral cortex; corpus striatum; dentate gyrus

ABSTRACT

AIM: To observe the protective effect of gypenosides (GP) on the neurons of hippocampus, cerebral cortex, corpus striatum, and dentate gyrus in cerebral ischemia-reperfusion injury of rats. **METHODS**: Modified 4-vessel occlusion (4-VO) method was used to establish the model of acute global ischemia. The acridine orange (AO) staining method was used to observe the DNA and RNA contents of cerebral ischemia-reperfusion injury model in the areas. **RESULTS**: The fluorescent intensity (reflecting DNA and RNA contents) of the DNA and RNA in the areas of cerebral ischemia-reperfusion injury was markedly abated compared with the normal control group. In the group of ig GP (100 mg/kg) it was enhanced compared with the model group and was the same as the normal control group. **CONCLUSION**: The injury of the DNA and RNA in the areas of ischemia-reperfusion model was decreased by GP.

INTRODUCTION

Gypenosides (GP) are the main effective ingredient of *Cucurbitaceae gynistemma pentaphyllum* (Thunb) Mak. Presently more than 80 kinds of saponins have been isolated from it and the chemical structures of saponin Ⅲ, Ⅳ, Ⅷ, and Ⅻ are simular to ginsenoside Rb_1, Rb_3, Rd, and F_2, respectively[1]. It has been reported that GP protect both cerebral ischemia-reperfusion injury in rat and acute incomplete cerebral ischemia in rabbits and can improve learning and memory ability of old rats[2,3]. So it is considered that the protective effect of GP on cerebral ischemic tissue is not only due to GP inhibiting the production of free radicals and decreasing lipid peroxides, but also due to GP's protective action on neuronal membrane and its beneficial effect on the improving the microcirculation[4]. At present there is no report about the effect of GP on the DNA and RNA of neurons in cerebral ischemia-reperfusion injury. In the present study, we investigated the effect of GP on DNA and RNA in hippocampus, cerebral cortex, corpus striatum and dentate gurus, in order to explore the mechanism of the protective effect of GP on ischemia-reperfusion injury at the molecular level.

MATERIALS AND METHODS

RATS Male wistar rats weighing 240 g to 260 g (Grade Ⅱ, Certificate № 2000-014) were supplied from the Experimental Animal Center of Tianjin.

Drug and reagents GP (content more than 90 %) was purchased from Huaguang Industry and Commerce Limited Company (Xi-an, China) and acridine orange (AO) was purchased from Sigma Chemical Co. All other reagents were of AR grade and from standard commercial sources.

Model of cerebral ischemia-reperfusion of rats Thirty rats were randomly divided into 3 groups: (1) The treatment group of cerebral ischemia-reperfusion pretreated with GP (100 mg/kg): Before ischemia, GP was given for 7 d. The rats were anesthetized with pentobarbital sodium (ip 40 mg/kg) and fixed on a stereotaxis frame. The skin of the rats was incised and paravertebral muscles were separated from the midline. Both vertebral arteries were electrocogulated after exposure of the alar foramen. Twenty-four hours later, superficial anesthesia was induced with ether. A ventral midline incision was made in the neck. Both common carotid arteries were freed from surrounding tissue and occluded by microartery clips for 30 min. Then the clips

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were loosened and the reperfusion was performed 1 h after the onset of ischemia[5]. (2) The model group of cerebral ischemia-reperfusion was pretreated with distilled water for 7 d. The operated model was the same as the treatment group. (3) Rats in sham-operated control group followed the same procedure as the model group but vertebral arteries were not electrocogulated and common carotid arteries were not occluded.

Sampling and sectioning After a 1-h reperfusion, the rats in the above 3 groups underwent pentobarbital sodium (40 mg/kg, ip) anesthesia and were fixed stereotaxically. They were thorectomised and perfused promptly with normal saline (100 mL to 150 mL) in the left ventricle followed by 4 % paraformaldehyde 500 mL (4 ℃). The brains were removed quickly and fixed with 4 % paraformaldehyde at 4 ℃ for 7 – 9 h. They were then soaked in 20 % sucrose solution for more than 12 h. The brains were cut into 50-μm slices with a thermostat cryomicrotome (Bright Co, English) and placed in 0.01 mol/L PBS.

Staining and microscopy The fixed slices were stained with AO for 2 – 3 min, washed with phosphate buffer for 1 min, differentiated with calcium chloride solution for 30 s, and then rinsed 3 times with phosphate buffer[6]. After that the slices were observed under a UIII fluorescence microscope (Nikon Co, Japan) and photographed.

RESULTS

Under fluorescence microscope, the DNA and RNA in the hippocampal CA_1 area and CA_3 area, cerebral cortex, corpus striatum, and dentate gyrus in the control group were observed with a yellow-green fluorescence homogeneously distributed in the pyramidal neuron layer of CA_1 and CA_3 areas, cerebral cortex and corpus striatum neuron layer, and the granular cells layer of dentate gyrus (Fig a1, b1, c1, d1, e1). Their borders were clear. In the ischemia-reperfusion model group, the fluorescent intensity of DNA and RNA (reflecting the content of DNA and RNA) in hippocampal CA_1 and CA_3 areas, cerebral cortex, corpus striatum, and dentate gyrus was significantly decreased (Fig a2, b2, c2, d2, e2). Their borders were not clear. In the group pretreated with GP (100 mg/kg), the fluorescent intensity of DNA and RNA in these regions was significantly enhanced (Fig a3, b3, c3, d3, e3), and the fluorescent reaction intensity, morphology, and distribution in the model were similar to the control group.

DISCUSSION

It has been reported that the neuronal injury of cerebral ischemia-reperfusion is a passive process of neuronal necrosis accompanied with apoptosis[7]. It has been generally accepted that hippocampal CA_1 and CA_3 areas and granular cells in dentate gyrus are ischemia-reperfusion vulnerable areas. Cerebral ischemia and reperfusion may lead to the whole destroyment of transcription and translation of the heat shock protein gene (HSP70 gene) in the neurons of hippocampal CA_1 and CA_3 areas and in the granular cells of dentate gyrus. HSP can alter cells and increase their resistance to anoxia to prevent further necrosis. Ischemia-reperfusion can increase intracellular calcium level and activate Ca^{2+}- and Mg^{2+}-dependent endonucleases by which DNA are cut into different base pair oligonuclcotides[8]. In our study, it has been shown that the fluorescent intensity of DNA and RNA in hippocampal CA_1 and CA_3 areas and dentate gyrus is significantly declined in the cerebral ischemia-reperfusion model group. It has been proved that the contents of DNA and RNA have decreased in the areas. The formation of DNA laddering is one of the characteristics of neuron apoptosis. GP have a protective effect on cerebral ischemia-reperfusion in rat hippocampus and dentate gyrus, thus declining the ischemia-reperfusion injury on DNA and RNA in these regions. In cerebral neuronal system, besides hippocampus and dentate gyrus, corpus striatum and cerebral cortex are also sensitive to ischemia and hence are ischemic vulnerable areas. Our present study has shown that GP have a protective effect on the neurons of cerebral cortex and corpus striatum in ischemia-reperfusion injury and then decrease the injury on DNA and RNA in these areas. Several studies have demonstrated that with an increase in ischemic time, iNOS (inductive nitric oxide synthetase) will be activated and large quantities of neurotoxic NO (having some effect on delayed neuron apoptosis) will be produced[9]. Only future studies will be able to clarify the mechanism on the effect of GP on anti-aging, improving learning and memory, and protecting cerebral ischemic tissue.

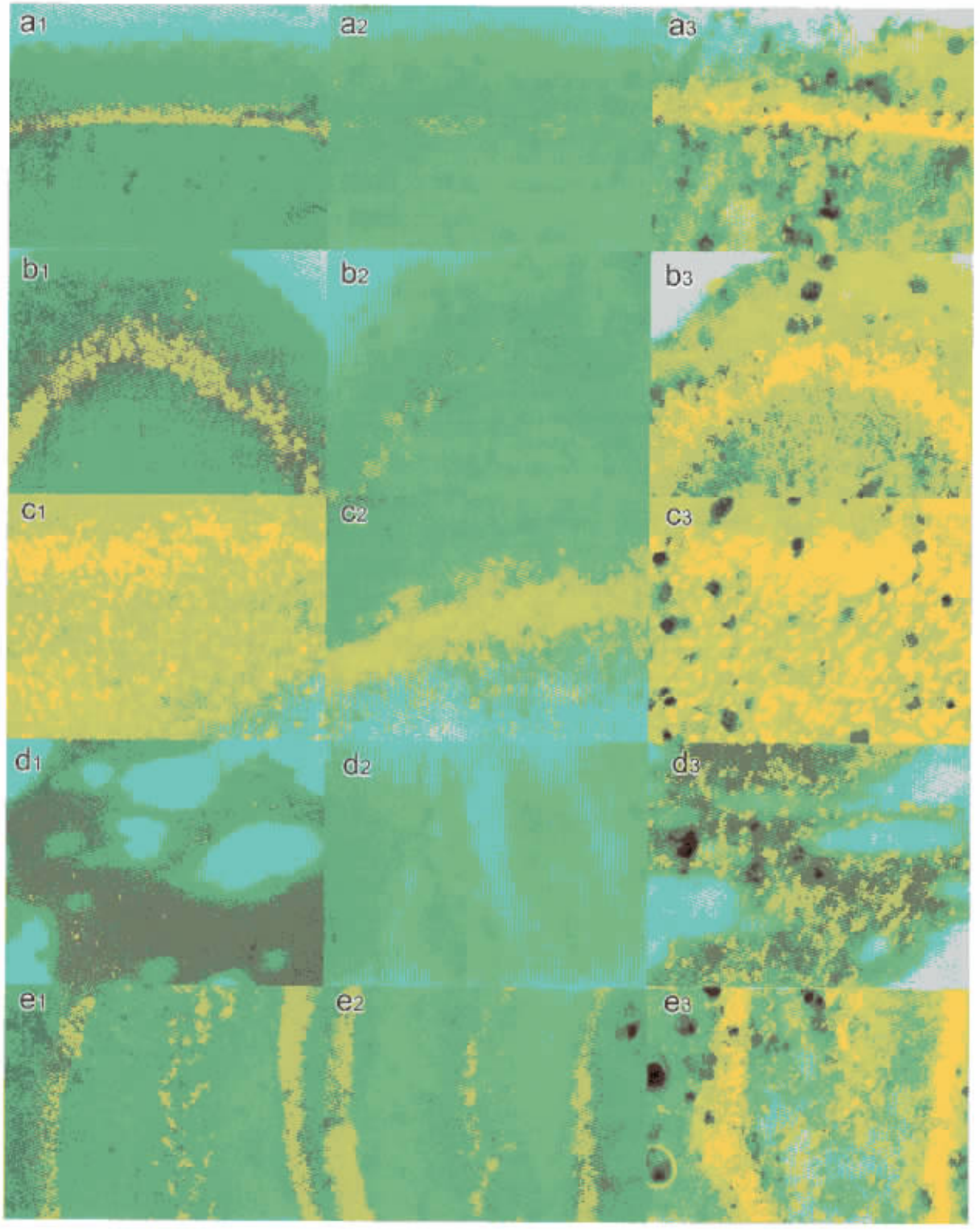

Fig 1. DNA and RNA staining in CA_1 area (a), CA_3 area (b), cerebral cortex (c), corpus striatum (d), and dentate gyrus (e). 1) the control group. 2) the model group. 3) the GP group. × 100.

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绞股蓝总皂苷对全脑缺血再灌注损伤大鼠神经元 DNA 和 RNA 的保护作用

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关键词 绞股蓝总皂苷；脑缺血；再灌注损伤；海马；大脑皮质；纹状体；齿状回

目的：观察绞股蓝总皂苷(GP)对全脑缺血再灌注大鼠海马、大脑皮层、纹状体及齿状回的保护作用. **方法**：采用 4-血管阻断(4-VO)方法建立大鼠急性全脑缺血模型，用吖啶橙染色法观察不同脑区 DNA 和 RNA 含量的变化. **结果**：与正常对照组比较，全脑缺血再灌注大鼠各脑区 DNA 和 RNA 吖啶橙染色后的荧光强度(反映 DNA 和 RNA 含量)明显减弱；GP 100 mg/kg ig 给药组各脑区 DNA 和 RNA 吖啶橙染色后的荧光强度强于全脑缺血再灌注模型组，与正常对照组相似. **结论**：GP 明显减轻缺血再灌注对大鼠海马、大脑皮层、纹状体及齿状回 DNA 和 RNA 损伤.

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