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

QI Gang1, ZHANG Li, XIE Wen-Li, CHEN Xiao-Yi, LI Ji-Sheng
( Department of Pharmacology, Medical College of CPAPF, Tianjin 300162, China)

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, VII, and X are similar to ginsenoside Rb1, Rb2, Rd, and F2, respectively. It has been reported that GP protect both cerebellar ischemia-reperfusion injury in rats and acute incomplete cerebral ischemia in rabbits and can improve learning and memory ability of old rats. 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 improving the microcirculation. 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 gyrus, 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 No 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 electrocoagulated 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

1 Correspondence to Dr QI Gang.
Received 2000-06-14 Accepted 2000-11-01
were loosened and the reperfusion was performed 1 h after
the onset of ischemia[5]. (2) The model group of cere-
bral ischemia-reperfusion was pretreated with distilled wa-
ter 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 electrocoagulated and
common carotid arteries were not occluded.

Sampling and sectioning After a 1-h reperfu-
sion, the rats in the above 3 groups underwent pentobar-
bital sodium (40 mg/kg, ip) anesthesia and were fixed
stereotaxically. They were thoracotomised and perfused
promptly with normal saline (100 mL to 150 mL) in the
left ventricle followed by 4 % paraformaldehyde 500 mL
(4 °C). The brains were removed quickly and fixed
with 4 % paraformaldehyde at 4 °C 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 ther-
mostat cryomicrotome (Bright Co, England) 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 so-
lution 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 CA1 area and CA3 area, cerebral cor-
tex, corpus striatum, and dentate gyrus in the control
group were observed with a yellow-green fluorescence ho-
mogeneously distributed in the pyramidal neuron layer of
CA1 and CA3 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 fluores-
cent intensity of DNA and RNA (reflecting the content of
DNA and RNA) in hippocampal CA1 and CA3 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, mor-
phology, and distribution in the model were similar to
the control group.

DISCUSSION

It has been reported that the neuronal injury of cere-
bral ischemia-reperfusion is a passive process of neuronal
tissue accompanied with apoptosis[7]. It has been gen-
erally accepted that hippocampal CA1 and CA3 areas
and granular cells in dentate gyrus are ischemia-reperfusion
vulnerable areas. Cerebral ischemia and reperfusion may
lead to the whole destruction of transcription and transla-
tion of the heat shock protein gene (HSP70 gene) in
the neurons of hippocampal CA1 and CA3 areas and in the
granular cells of dentate gyrus. HSP can alter cells and
increase their resistance to anoxia to prevent further necro-
sis. Ischemia-reperfusion can increase intracellular calcium
level and activate Ca2+- and Mg2+-dependent en-
donucleases by which DNA are cut into different base pair
oligonucleotides[8]. In our study, it has been shown that
the fluorescent intensity of DNA and RNA in hippocampal
CA1 and CA3 areas and dentate gyrus is significantly de-
clined 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 ladd-
ering is one of the characteristics of neuron apoptosis.
GP have a protective effect on cerebral ischemia-reperfu-
sion in rat hippocampus and dentate gyrus, thus declining
the ischemia-reperfusion injury on DNA and RNA in
these regions. In cerebral neuronal system, besides hip-
campus 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 in-
crease in ischemic time, iNOS (inductive nitric oxide syn-
thesetase) will be activated and large quantities of neu-
rotoxic NO (having some effect on delayed neuron apop-
tosis) 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. $\times100$. 
REFERENCES