The Effect Of GinsenosideRb1 on IL-1β in Focal Cerebral Ischemia/Reperfusion Injury Model of Rats

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Introduction: Mechanisms of cerebral ischemia-reperfusion injury (I/R) is very complicated. It is a complex cascade of multiple factors involved, such as inflammation, calcium overload, and oxygen free radicals. Recent studies have found that inflammation involved in ischemic brain injury, as one of its important pathological mechanisms. Therefore, inhibition inflammation after ischemia may be an effective method for the treatment of cerebral ischemic injury. Ginsenoside Rb1 is one of the active ingredients of ginseng, a representative component panaxadiol type. Studies have shown that ginsenoside Rb1 have a protective effect on central nervous system diseases , The main mechanism of action include: inhibition of calcium overload, inhibition of neuronal apoptosis, and free radical scavenging antioxidant. In this study, we used middle cerebral artery occlusion (MCAO) I/R model to further investigate the effects of ginsenoside Rb1 on rats cerebral I/R and inflammation-related factors IL-1β , to provide more adequate experimental basis for ginsenoside Rb1 treatment on cerebral vascular disease.

Materials and methods: I/R model preparation. Rats in each group were made into the I/R model referring to the modified Longa EZ method [1] by suturing the external carotid artery. When finished the right side of the MCAO model , recorded the moment of time, the internal carotid artery embolization after 120 min, pull the suture to restore cerebral blood flow. Reference to an animal awake Longa EZ [1] neurological defects 5 of 5 stars law behavior of rats were scored from 0 (normal) : no dysfunction ; 1 (mild neurobehavioral defects): not completely stretch the left forelimb; 2 points (moderate neurobehavioral defects): rotate to the left; 3 points (severe neurobehavioral defects): to the left side of the dump; 4 (very severe neurobehavioral defects): no independent activity with sense suppression. Score 1 point or more were used as experimental subjects, while 0 were removed.

Neurological deficit scores. After 24 h ofcerebral ischemia and reperfusion, we used a single blind method to score, refering to Longa EZ [1] 5 degrees and 4 points system.

Infarct volume measurement. Brain tissue was removed from the freezer, the spacer 5 into 2 mm coronal sections continuous with 2% TTC staining buffer in the dark 30 min, MI portion is dyed white. The sum of the area of the infarct volume after application of a digital camera to take pictures IPP6.1 image analysis software to calculate the area of infarction after then multiplied by 2 mm.
Detection of brain tissue and serum IL-1β expression. After paraffin sections 3% H2O2 for 10 min at room temperature to inactivate endogenous peroxidase citrate buffer (PBS) 3 min ions 3% H2O2

for 10 min at room temperature to inactivate endogenous peroxida℃, 3 min repair natural cooling,

PBS 3min n at room temperature to inactivate endogenous peroxidase citrate calculate the area body overnight at 4℃ covering tissue sections rewarming 30min, plus mouse anti-rabbit secondary antibody, PBS 3 min se citrate calculate the area body overnight at 4 then multiplit, under mounted, optical microscope, and negative photos with PBS solution instead. Positive cells under a microscope as brown, recorded and compared among groups of brain tissue IL-1β positive cells. Detection of serum IL-1β: arterial blood 8ml, adding tubes containing heparin, mixing at 4℃ 3000r/min centrifuged 10 min, the serum spare. Double antibody sandwich ELISA assay operating strictly according to kit instructions steps.

Results:

1. Ginsenoside Rb1 reduces neurological deficit scores and infract volume of MCAO rats.

Table 1. Nerve impairment scores and infarct volume ($\bar{x}\pm s$)

ng/kg)		volume（mm^3）
roup	**	**
group		58.8
se Rb1		27.0**
n dose Rb1	*	32.4**
ose Rb1	**△	5.7**△

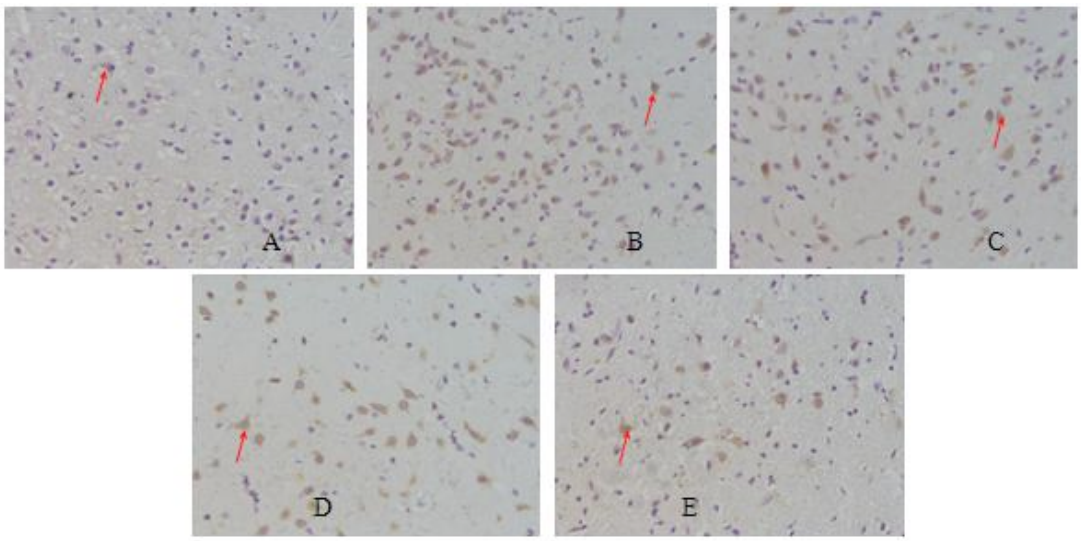
Note: Compared with model group, * P <0.05, ** P <0.01; compared with low-dose ginsenoside _ group, △ P <0.01

2. Ginsenoside Rb1 decreases IL-1β-positive cells and serum IL-1β concentrations.

Table 2. IL-1β immunohistochemistry of brain tissue and serum ($\bar{x}\pm s$)

oups)	ositive cells	L-1β contents（pg/mL）
am group	±1.7**	±2.8**
odel group	±6.3	±35.4
se Rb1	)±3.7*▲	±27.1**
n dose Rb1	±2.5**	±19.1**
ose Rb1	)±2.3**	±24.0**△

Note: Compared with model group, * P <0.05, ** P <0.01; compared with low-dose ginsenoside _ group, △ P <0.05



Note: A sham group; B as a model group; C as _ low-dose group; D as _ dose group; E is _ high-dose group; red arrows brown cells IL-1β positive cells

Fig 1. Brain tissue IL-1β-positive cells of each group (DAB stain, × 400)

Discussion: Pathogenesis of ischemic cerebrovascular disease is due to a sudden interruption of blood flow or decreased , early thrombolytic therapy , there was a recovery after ischemic area blood supply is more severe brain damage , which is called cerebral I / R. Researches found that its pathogenesis is a multi- factor , multi-link cascade . Recent studies have found that inflammation in a variety of inflammatory cytokines such as IL-1β, TNF-α and other involved in ischemic brain injury "waterfall effect" , as one of its important pathological mechanisms [2,7] . After Yamasaki Y et al [8] reported that intraventricular injection of recombinant IL-1β increased cerebral I / R infarct volume showed that IL-1β protein is an important pathological mechanism in rat brain I /R 's . Hara F et al[9] showed that selective inhibition of IL-1β can reduce cerebral I / R, tips IL-1β levels in measuring cerebral I / R has important significance. In this experiment, the rats with cerebral ischemia reperfusion after 2 h 24 h, ischemia and immediately injected intraperitoneally with different doses (20,40,80 mg / kg) ginsenosides Rb1, to varying degrees can reduce neurological deficits in rats severity score and infarct volume , inhibits the expression of IL-1β protein in the brain , reducing blood IL-1β levels . Ginsenoside Rb1 in rats showed that I / R has a protective effect and its inhibition of inflammatory cytokines IL-1β protein. This is probably one of the remission of cerebral ischemia-reperfusion injury in an important mechanism of ginsenoside Rb1.

Ginseng is a traditional Chinese medicine, used in clinical medicine which has 2000 years of history . Ginsenoside Rb1 is the active ingredient extracted from ginseng , is one of the active ingredients of PDS . Ginsenoside Rb1 with anti- lipid peroxidation, scavenging free radicals , inhibiting the release of excitatory amino acids and anti-apoptosis , inhibition of the inflammatory response , such as a variety of roles . Studies have shown that the central nervous system diseases , ginsenoside Rb1 can be achieved through a variety of mechanisms of brain protection. Lim JH and Huang Yi Sen [10,11]. The study also showed that the protective effect of ginseng saponin Rb1, and Panaxatriol brain and scavenging free radicals and inhibiting lipid peroxidation . Jiangshan et al [12] in the study of ginsenoside Rb1 on rat hippocampal slices observed protective effect of ischemic injury to the extent of ginsenosides Rb1 can promote recovery potential along the peaks of the hippocampal slices under

ischemic conditions , indicating that ginsenosides Rb1 confrontation GLu excitotoxic effect. Wu XM et al [13] found that ginsenoside Rb1 can reduce TNF-α, VCAM-1 expression in human umbilical vein endothelial cells , suggesting that ginsenoside Rb1 on cardiovascular disease has clinical value . These findings are consistent with the study. In addition, the results also found that different doses of ginsenoside Rb1 group has a protective effect , with the low-dose group, the high-dose group rat nerve impairment score , infarct volume, differences in brain tissue content and serum IL-1β were statistically significant , but the medium-dose group compared with the low-dose group, the difference was not statistically significant , suggesting that the high -dose group ginsenoside Rb1 (80 mg / kg) of the brain protective effect is superior to low dose and dose group .

In summary, the present study the classical rat brain I / R model , different concentrations of ginsenosides Rb1 treatment was observed 2 h after cerebral ischemia and reperfusion 24 h neurological deficit scores , infarct volume and serum IL-1β found to give ginsenoside Rb1 changes in brain tissue of rats and serum levels of IL-1β showed that ginsenoside Rb1 can suppress inflammation by regulating IL-1β levels , cerebral I / R. For other inflammatory factors, such as NF-κB, TNF-α , etc., without making detection of the experiment , so whether ginsenoside Rb1 inflammation but also through other pathways play a protective role in the brain , but also the need for further research .