Effect of curcumin on diabetic rat model of cerebral ischemia

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Abstract: To investigate the effect of curcumin on cerebral ischemia in diabetic rats the effects and features. intravenous injection alloxan diabetes model, to give alloxan first seven days the tail measured blood glucose value, the election successful model rats were fed with large, medium and small doses of curcumin suspension, Shenqijiangtang suspension and the same volume of saline, administered once daily. The first 10 days after administration 2h (fasting 12h) rat tail vein blood glucose values measured in the first 20 days after administration of 2h (fasting 12h), do cerebral ischemia surgery; rapid carotid artery blood after 30min rats were decapitated, blood serum, blood glucose and glycated serum protein levels; take part of the brain homogenates plus nine times the amount of normal saline, made 10 percent of brain homogenates. Another part of the brain tissue, in the light microscope observation of pathological tissue. Compared with model group, large, medium and small doses of curcumin can significantly lower blood sugar and glycated serum protein levels, significantly reduced brain homogenates lactic acid content and lactate dehydrogenase activity; large, medium-dose curcumin can significantly increase brain homogenates Na^{+}-K^{+}-ATP activity, dose curcumin can significantly improve brain homogenates Ca^{++}-Mg^{++}- ATP activity. Curcumin can reduce blood sugar in diabetic rat model of cerebral ischemia and improve brain energy metabolism, improve their brain tissue resistance to ischemia and hypoxia, cerebral ischemia in diabetic rats have a good drop the role of sugar and protect brain tissue

Keywords: Curcumin; Diabetes; Cerebral ischemia.

INTRODUCTION

At present, the incidence of diabetes is rising year by year, and the age of onset is increasingly younger age. Mortality of diabetes is only below that of the accidental death and cancer death, although a lot of west medicines treat diabetes, but most can't cure, and many side effects. Therefore, people now pay more attention to some of the research on Chinese and JiangtangKeli plants, use it for the adjuvant treatment of diabetes (Wang *et al*., 2014). This experiment observed the effects of curcumin on rat model of diabetic cerebral ischemia and to explore its mechanism of action.

MATERIAL AND METHODS

Materials animals

SD150 male rats, weighing 160~180g, clean grade, Hebei Experimental Animal Center, Certificate of Conformity: DK0509011. Animal feeding ambient temperature (25± 3□), humidity (55±10)%, animal free diet, drinking water.

Drugs and reagents

Curcumin, the natural pigment Ltd. Henan wide Ye provided content greater than 90% (batch); alloxan, U.S. sigma company (batch); Shenqijiangtang, Shandong Lunan thick S & P pharmaceutical Co., Ltd. (lot number 0504505); saline, from Zhengzhou chemical pharmaceutical Co (lot 040409); ATP detection kit, Nanjing Jiancheng Bioengineering Institute production (Lot 20051222); LD detection kit, Nanjing Jiancheng Institute of Bioengineering production (batch number 20051222); LDH assay kit, Nanjing Jiancheng Production (Batch Number 20051222), Institute of Biological Engineering; Glycated serum protein, Nanjing Jiancheng Bioengineering Institute production (Lot 20051222); glucose assay kit, Zhejiang East gull biological Engineering Co., Ltd. (batch 2005090140); Coomassie blue protein assay kit, Nanjing Jiancheng Bioengineering Institute Production (Lot 20051222).

Instrument

TGL-16G high-speed refrigerated centrifuge, Shanghai Anting Scientific Instrument Factory production; adjustable pipette, the Hai Leibo Analytical Instruments Co., Ltd.; UV-2000 spectrophotometer, UNICO (Shanghai) Instrument Co., Ltd; homogenizer, Ningbo Chi Biotechnology Co., Ltd. production.

Methods

Grouping intervention: Take 150 male SD rats, weighing 160~180g, 10 rats were randomly selected as a control group, rats were fasted for 12h after intravenous injection of alloxan 40mg• kg-1 (0.2mL• 100g-1) made diabetic model, the control group does not give alloxan, only intravenous injection of the same volume of saline. Alloxan in to the first seven days the tail blood glucose values measured, select blood glucose>11mmol•L-1 and has obvious polydipsia, polyphagia, 50 urinary symptoms in rats, according to a random blood glucose were divided into five groups, large, medium and small doses of curcumin group Shenqijiangtang model group. Large, medium and small doses of curcumin group: respectively 2mL• 100g-1 dose orally curcumin solution (saline paired

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with curcumin 10mg• mL-1, 5mg• mL-1, 2.5 mg• mL-1). Shenqijiangtangs: Press 2mL• 100g-1 gavage Shenqijiangtang suspension (with saline paired 25 mg• mL-1). Model group and the control group: press 2mL• 100g-1 normal saline. Above were administered starting from the first seven days, administered once a day for

Consecutive 20 days, the first 10 days gavage for 2 h (fasted for 12h) rat tail vein blood glucose values measured. 2h (fasted for 12h) 10% chloral hydrate 0.35mL• 100g-1 intraperitoneal injection of anesthesia, as a neck incision, separate bilateral carotid artery in the first 20 days after dosing, each set to a suitable artery length of surgical thread, ligation of blood vessels, which in addition to the control group without bilateral carotid artery ligation, the other operations with modeling groups. After a quick carotid artery blood 30 min after rats were decapitated, blood serum, according to test kit instructions to measure blood sugar and glycated serum protein levels; dissected the brains, plus nine times the amount of normal saline homogenized, made 10% of the brain homogenized; another part of the brain to do tissue morphology.

STATISTICAL ANALYSIS

Data was analyzed by SPSS 11.5 for windows statistical package for statistical analysis of medical data, and were statistically processed by the first author, measurement data expressed as mean ± standard deviation ($\bar{x}$ ±s), comparison between groups analysis of variance (LSD); rating data using Ridit test.

RESULTS

Experimental analysis of the number of animals included 60 rats, in the final analysis, without any loss values

In addition to the control group, blood glucose levels before administration of each group were not significantly different, indicating grouping uniform. Compared with the corresponding control group at the beginning of the first 10 days, the first 20 days of blood glucose were significantly higher in the model group (P<0.01), making diabetes model successfully explained. Compared with the model group, high-dose curcumin group and Shenqi Jiangtang Keli group can significantly reduce blood sugar

Table 1: Curcumin on blood glucose levels in diabetic cerebral ischemia model in rats ($\bar{x}$ ±s)

Group	Animal quantity (Pc)	Dose (g/kg)	Original Blood sugar (mmol/L)	Blood sugar in 10th day (mmol/L)	Blood sugar in 20th day (mmol/L)
Control group	10	□	5.144±0.657**	5.236±0.498**	5.269±0.465**
Model group	10	□	21.890±4.673	19.171±4.256	16.036±4.426
Shenqi Jiangtang Keli group	10	0.5	19.086±5.737	13.207±3.088**	11.557±2.119**
High-dose group of curcumin	10	0.2	19.262±5.265	13.998±2.949**	11.215±2.075*
Middle-dose group of curcumin	10	0.1	20.354±6.040	15.605±2.869*	12.294±2.108*
Small-dose group of curcumin	10	0.05	20.829±5.619	15.791±3.263*	12.711±2.246*

Table 2: Effect of Curcumin on rats with diabetic cerebral ischemia serum levels of glycated serum protein model (± s)

Group	Animal quantity (Pc)	Dose (g/kg)	Glycated Serum Protein
Control group	10	□	0.795±0.052**
Model group	10	□	1.390±0.157
Shenqi Jiangtang Keli group	10	0.5	0.969±0.164**
high-dose group of curcumin	10	0.2	0.908±0.064**
Middle-dose group of curcumin	10	0.1	1.010±0.043**
Small-dose group of curcumin	10	0.05	1.047±0.117**

Table 3: Effect of Curcumin on rat model of diabetic ischemic brain homogenates lactic acid content and lactate dehydrogenase activity ($\bar{x}$ ±s)

Groups	Animal Quantity (Pc)	Dose (g/kg)	Lactate (mmol/gprot)	Lactate dehydrogenase (U/gprot)
Control group	10	□	1.108±0.218**	11966.0±2595.1**
Model group	10	□	1.985±0.301	14974.3±1198.5
Shenqi Jiangtang Keli group	10	0.5	1.627±0.405**	13249.3±782.9**
high-dose group of curcumin	10	0.2	1.350±0.208**	12791.9±503.7**
Middle-dose group of curcumin	10	0.1	1.327±0.312**	12800.3±1824.2**
Small-dose group of curcumin	10	0.05	1.401±0.351**	12950.2±1210.6**

*Compared with the model group P<0.05, ** compared with model group P<0.01

levels in rats (P<0.01) in the first 10 days, 20 days, in middle and small doses curcumin can significantly lower blood sugar levels (P<0.05).

Compared with the control group, the model group were significantly elevated glycated serum protein levels (P<0.01), shows glucose metabolism, diabetes model made successful. Compared with the model group, large, medium and small doses of curcumin group and Shenqi Jiangtang Keli group can significantly reduce glycated serum protein levels (P<0.01)

Compared with the control group, model group brain homogenates lactic acid content and lactate dehydrogenase activity were significantly increased (P<0.01), shows the brain tissue hypoxia. Compared with the model group, large, medium and small doses curcumin group and Shenqi Jiangtang Keli group can significantly reduce brain homogenates lactic acid content and lactate dehydrogenase activity (P<0.01).

Effects of curcumin on cerebral ischemia in diabetic rats brain homogenates activity of ATP in table 4

Compared with control group, model group brain homogenates Na^+-K^+-ATP activity and Ca^{++}- Mg^{++}- ATP activity were significantly lower (P<0.01), shows reduced brain energy metabolism. Compared with the model group, large, medium-dose curcumin group can significantly improve brain homogenates Na^+-K^+-ATP activity (P<0.01), the middle dose curcumin group can significantly improve brain homogenates Ca^{++}-Mg^{++}- ATP activity (P<0.01).

Effect of Curcumin on cerebral ischemia model of diabetic nerve cells and glial cells in brain atrophy in table 5

Compared with the control group, cells, cortical glial cells, nerve cells and brain nerve group glial cell groups were significant pathological changes in cortical model group was significantly shrunken (P<0.01), cerebral ischemia model description made success. Compared with the model group, large, medium-dose curcumin group can significantly reduce cortical neurons, cortical glial cells, nerve cells and brain nerve Mission Mission glial cells atrophy (P<0.01); small doses of curcumin group can significantly reduce cerebral cortex nerve cells, nerve cells, brain atrophy group (P<0.05), can significantly reduce the glial cells of the brain atrophy group (P<0.01); Shenqi Jiangtang Keli group can significantly reduce cerebral cortical neurons, Mission brain nerve cells, glial cells in brain atrophy group (P<0.01), can significantly reduce the cortical glial cells atrophy (P<0.05).

Each group of brain tissue morphology (Annex1, 2): control group cortex and brain cell mass of nerve cells and glial cells no abnormal pathological changes were

Table 4: Effects of Curcumin on cerebral ischemia in diabetic rats brain homogenates activity of ATP ($\bar{x}$ ±s)

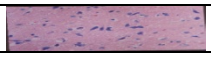
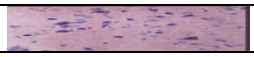
Groups	Animal quantiy (Pc)	Dose (g/kg)	$Na^{\square}$-$K^{\square}$-ATP (μmol/mgprot/hour)	$Ca^{\square\square}$-$Mg^{\square\square}$-ATP (μmol/mgprot/hour)
Control group	10	□	1.639±0.780**	1.268±0.508**
Model group	10	□	0.737±0.290	0.526±0.270
ShenqiJiangtangKeli group	10	0.5	0.904±0.183	0.822±0.572
High-dose curcumin group	10	0.2	1.193±0.362**	0.825±0.413
Middle-dose curcumin group	10	0.1	1.319±0.549**	1.097±0.397**
Small-dose curcumin group	10	0.05	0.810±0.204	0.746±0.264

Table 5: Effect of Curcumin on cerebral ischemia model of diabetic nerve cells and glial cells in brain atrophy ($\bar{x}$ ±s)

Groups	Animal quantity (Pc)	Dose (g/kg)	Cortical neurons	Cortical glial cells	Cranial nerve cell group	Mission brain glial cells
Control group	10	□	1.67±0.24**	2.48±0.76**	0.74±0.19**	0.82±0.27**
Model group	10	□	86.35±11.63	26.74±8.35	93.68±21.76	56.38±11.28
Shenqi Jiangtang Keli group	10	0.5	23.74±8.6**	17.62±2.36*	48.27±8.96**	38.54±12.64**
high-dose group of curcumin	10	0.2	3.87±1.34**	8.68±3.75**	2.83±2.68**	6.58±2.37**
Middle-dose group of curcumin	10	0.1	45.38±17.65**	11.27±11.62**	47.28±11.37**	25.77±10.26**
Small-dose group of curcumin	10	0.05	70.43±21.32*	18.34±10.50	70.39±23.78*	29.16±18.61**

*Compared with the model group P<0.05, **compared with model group P<0.01

Annex 1 Effects of curcumin on diabetic cerebral ischemia model of cortical neurons and glial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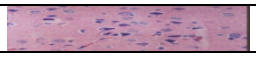
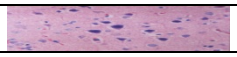
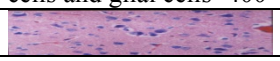
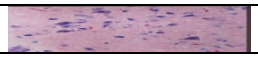
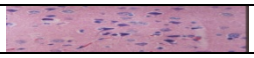
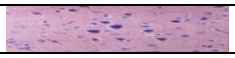
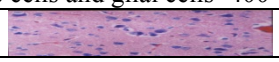
		
High-doescurcumin group diabetic nerve cells and glial cells×400	Middle-dosecurcumin group diabetic nerve cellsandglial cells ×400	Small-dose curcumin group diabetic nerve cells and glial cells×400

Annex 2 Effects of curcumin on cerebral ischemia in diabetic nerve cells and glial group

		
ControlGroup diabetic nerve cells andglial cellss×400	Model Group diabetic nerve cells and glial cells×400	Shenqijiangtangkeli group diabetic nerve cells and glial cells×400
		
High-doescurcumin group diabetic nerve cells and glial cells×400	Middle-dosecurcumin group diabetic nerve cellsandglialcells×400	Small-dose curcumin group diabetic nerve cells and glial cells×400

normal; nerve and brain cortex model group corporation cells and glial cells were found reduced brain cell volume, cell membrane and nuclear membrane shrinkage phenomenon, glial cells showed shrinkage and other pathological changes; Shenqi Jiangtang Keli group and brain cortex groups of nerve cells and glial cells most of the pathological changes significantly restored, a small part of the nerve cells and glial cells is still shrinking, karyopycnosis like; large doses of curcumin pathological changes in brain cortex and brain cell mass of nerve cells and glial cells majority obviously been restored, but the individual nerve and glial cells do not recover, there is still some shrinkage; dose curcumin group part of the brain cortex and glial cells and nerve cell clusters of nerve cells has been significantly restored, some still shrinking; Small doses of curcumin group and a small part of the brain cortex cell clusters of nerve cells and glial cells have been restored, the majority of nerve cells and glial cells continued to show a contraction.

DISCUSSION

Diabetes is an important means to study animal models of diabetes, there are a variety of modeling methods, which use alloxan modeling is one of the commonly used methods. Alloxan mouse pancreatic β cells can destroy established mouse model of diabetes, elevated blood sugar cause diabetes model to achieve, but also because of the tissue toxicity, good stability, high survival rate of animals become used more at home and abroad one method for preparing an animal model of diabetes (Du *et al*., 2009). Therefore, it can be used to establish diabetes model by injection of alloxan.

Curcumin has been reported for the treatment of diabetes and its complications have a good effect, can significantly reduce the activity of SOD and CAT experimental diabetic rats, MDA content increased significantly, effectively improve blood sugar levels in type 2 diabetes and reduce the amount of urine; reducing urinary albumin excretion and urinary TXB2 excretion (Chen *et al*., 2009).

Recent studies have demonstrated that curcumin reduced infarct size, reducing inflammation, lipid peroxidation and anti-free radicals, into the recovery of neurological function, in addition to free radicals, anti-oxidation and anti-apoptosis (Li *et al*., 2012). Our previous studies have shown that curcumin for diabetes and cerebral ischemia have a good effect.

This experiment compared with the control group, model group LD and LDH increased significantly, significantly reducing ATP enzyme activity, have significant differences, indicating that successful model of cerebral ischemia. Curcumin treatment group but decreased production of lactic acid, lactic acid dehydrogenase activity was reduced, but a significant increase in ATP activity of curcumin on cerebral ischemia has a protective effect, which may improve brain energy metabolism, improve brain tissue missing blood hypoxia resistance, increase energy supply of cells, reduce mitochondrial damage.

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