Effect of *baicalein* with different concentrations on myocardial ischemia reperfusion injury in rats

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Abstract: *Baicalein* is a kind of flavonoids. Long term use of flavonoids can reduce blood pressure by releasing physiological NO to keep the vascular endothelial function of hypertensive animals and reduce myocardial ischemia injury. The purpose of this study is to explore the effect of baicalein on myocardial I/R damage and explore its mechanism of action, so as to provide experimental evidence for treatment. The results show that baicalein has a significant improvement in I/R damage, mainly in reducing myocardial infarction rate, reducing the release of myocardial isozyme LDH and CK, reducing the level of oxidative stress and reducing the apoptosis of cardiac myocytes. In the *baicalein* group, the myocardial ischemia state of isolated rat hearts was improved. Among them, myocardial infarction rate in low and middle concentration group was significantly decreased ($P<0.05$ or $P<0.01$), and there was no significant change in high concentration group ($P>0.05$). The apoptosis rate of cardiac myocytes in the isolated group of *baicalein* was significantly decreased ($P<0.01$). The role of *baicalein* in the cardiovascular system needs further test and clinical research.

Keywords: *Baicalein*, myocardial ischemia, apoptosis rate, antioxidation, drug mechanism.

INTRODUCTION

In recent years, the incidence of ischemic cardiomyopathy is increasing. In most cases, the ischemic myocardium gets blood perfusion in time and the myocardium tissue will be repaired to some extent, and the heart function will be restored and the patient will get better. Sometimes this kind of reperfusion not only can not restore the cardiac function, improve the condition, but will appear obvious dysfunction and structural damage (Anding *et al.,* 2015; Benson *et al.,* 2017). It is called myocardial ischemia reperfusion Injury (MIRI), which is aggravated or even irreversibly damaged on the basis of myocardial ischemia. MIRI is a complex and multifactorial pathophysiological process. At present, the mechanism of MIRI has not been fully elucidated. It is generally believed that free radical outbreak, calcium overload, cardiac energy metabolism disorder, endothelial cell dysfunction, neutrophil infiltration, cell apoptosis and mitochondrial damage are important reasons for the development of MIRI during myocardial reperfusion (Eugene *et al.,* 2016).

Baicalein is one of the active ingredients extracted from *Scutellaria baicalensis georgi*. Studies have shown that baicalein can reduce myocardial ischemia/reperfusion injury in rats, and significantly inhibit arrhythmia induced by ischemia/ reperfusion injury (Anding *et al.,* 2015; Benson *et al.,* 2017). With the popularization and application of advanced medical technology such as coronary artery bypass and heart transplantation, diseases that have long been affected by human health have been well treated (Bergmann *et al.,* 2016). But during the above treatment, ischemia/reperfusion (I/R) damage often occurs, which seriously affects the therapeutic effect. I/R damage is a major obstacle to the efficacy of cardiovascular surgery (Chen *et al.,* 2009). In the whole process of myocardial I/R, severe myocardial tissue changes, cardiac function damage and metabolic abnormalities are often accompanied (Inzucchi *et al.,* 2015). In clinic, a series of diseases such as severe cardiac insufficiency, sudden decrease of blood pressure, arrhythmia and sudden death have worsened (Dindo *et al.,* 2004). At the time of myocardial ischemia/hypoxia, *baicalein* is combined with its receptor to regulate cell signaling pathway to affect the proliferation, differentiation and apoptosis of cardiac cells, and to play the role of myocardial protection.

Flavonoids are a kind of polyphenols which are widely used in plants. More than 5000 flavonoids have been found (Eugene *et al.,* 2016). The antioxidant effect of flavonoids is very significant in vitro. Flavonoids have many biological functions, including vasodilatation, anti-inflammatory and anti platelet aggregation. Studies have shown that flavonoids have a significant protective effect on myocardial I/R damage in isolated hearts (Emir *et al.,* 2014). *Baicalein* is a compound isolated from Scutellaria baicalensis Georgi. It can be divided into flavonoids according to its chemical structure (Hou *et al.,* 2015). *Baicalein* has significant antioxidant effects and certain anti-inflammatory and diuretic effects, and is often used in the treatment of various diseases (Ghoneum *et al.,* 2015). In recent years, researchers at home and abroad have increased the study of *baicalein*. A lot of studies have found that *baicalein* has a certain therapeutic effect

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on ischemic heart and cerebrovascular diseases (Jiahua *et al.,* 2011). Studies have shown that *baicalein* can reduce the expression of acetylcholine (ACE), thereby reducing brain I/R damage (Hu, 2013; Altorki *et al.,* 2016). The study shows that flavonoids have a significant protective effect on myocardial I/R damage in isolated heart *(*Jean *et al.,* 2017). This paper aims to explore the effect of baicalein on myocardial I/R damage and to explore its mechanism to provide experimental basis for the use of baicalein in the treatment of myocardial I/R injury.

MATERIALS AND METHODS

Drugs and reagents

The instrument includes Radnoti isolated heart perfusion device, 4S AD Instruments multichannel physiologic instrument.The drugs include baicalein (purity: > 99%); the reagents include superoxide dismutase (SOD), malondialdehyde (MDA), reduced glutathione / oxidized glutathione (GSH/GSSG), creatine kinase (CK), lactate dehydrogenase (LDH), interleukin 6 (IL-6), and swelling tumor necrosis factor α (TNF- α) reagents, paraformaldehyde, 25% glutaraldehyde, acrylamide and anhydrous ethanol.

Experimental animals and methods

50 adult Wistar male rats, weighing 280~380 g, were provided by Jining Medical University laboratory animal research center. The animal experiment program has been approved by the experimental animal ethics committee, which conforms to the principles of animal protection, animal welfare and ethics, and conforms to the relevant provisions of the national laboratory animal welfare ethics, No.FEJMUPF/16SQ.

Langendorff method was used to establish myocardial I/R injury model. The isolated hearts of 50 rats were divided into 5 groups randomly, with 10 rats in each group, namely, normal group, model group and baicalein high concentration group (45μmol/ml), medium concentration group (15 μmol/ml), low concentration group (3 μmol/ml). The dosage of baicalein was set according to pre experiment and acute toxicity test.The rats in the normal group were perfused with K-H fluid for 95 min, while the model group was perfused with K-H solution for 30 min, 20 min for reperfusion and 45 min for reperfusion. The baicalein administration group was perfused with K-H solution for 20 min, perfusion of baicalein containing K-H solution for 10 min, stopping irrigation for 20 min, and reperfusion of K-H solution for 45 min.1 ml of coronary effluents were collected before 10min, 20min and 40min after reperfusion in each group, respectively, and preserved in the refrigerator at -4°C before determination in order to prevent the degradation of the enzyme. All LDH and CK activities tests were carried out in strict accordance with the instructions of the kit.

After the end of the perfusion, wash the heart with phosphate buffer (PBS) and quickly put the heart in a refrigerator at 20°C for 5 min. Then, along the direction parallel to the heart atrioventricular groove, the left ventricle was cut into thin slices of equal thickness (1 to 2mm). The cut slices were put into 1%TTC dye solution and placed in a 37 degree water bath. The temperature was 15~30 min. After the reaction was completed, the myocardium slices were taken out in 10% formalin solution for about 24 h, then removed and placed on the appropriate background and scanned with a scanner. The non infarcted area was brick red and the infarct area was gray. The plane area quadrature method was used to calculate the myocardial infarction rate: myocardial infarction rate (%) = (infarct area / left ventricular area) x 100.

Numerical detection

The myocardial tissue of each group was made into 10% homogenate and operated strictly according to the instructions of SOD, MDA and GSH/GSSG kit. The activity of SOD, the content of MDA and the ratio of GSH/GSSG in the myocardium of rats were detected. According to In Situ Cell Death Detection Kit and POD kit instructions, cardiomyocyte apoptosis was detected. After dewaxing and hydrating, the slices were treated by 10mmol protease K for 15 min, and the slides were immersed in the reaction mixture of TUNEL, and 60 min was incubated at 37°C. The POD reaction solution was used to avoid light and incubate 30 min, which showed that the blue nucleus was stained into normal cells and the brown nucleus was stained to apoptotic cells (positive cells). The slides were analyzed by optical microscope. For each sample, 8 regions were randomly selected to count and calculate the average value. The apoptosis rate (%) of cardiac myocytes was equal to the total number of mean positive cells / the total number of cells was 100%.

STATISTICAL ANALYSIS

SPSS 10 software was used for statistical analysis. The measurement data were expressed as x±s, and t test was used for comparison between groups. $P < 0.05$ indicated that the difference was statistically significant.

RESULTS

Effects of baicalein on CK and LDH activity in isolated coronary artery effluents of rats

The activity of LDH and CK in the left ventricular outflow fluid of normal rats was very low, and the activity was kept constant at 10 min before reperfusion, and at 3 time points of 20 and 40 min reperfusion. Compared with the normal group, the activity of LDH and CK in the coronary outflow fluid increased significantly after reperfusion for 20 and 40 min in the model group ($P < 0.01$). Compared with the model group, the activity of

LDH and CK in the coronary effluent after 20 and 40 min reperfusion in the low and middle concentration group of baicalein group was significantly decreased (P < 0.05 or P <0.01), and there was no significant change in the high concentration group (P>0.05). The results showed that baicalein could inhibit the release of LDH and CK from myocardial cell injury, as shown in table 1.

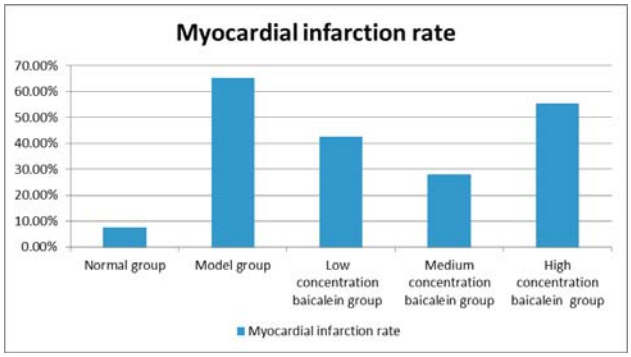

Fig. 1: Detection results of myocardial infarction rate

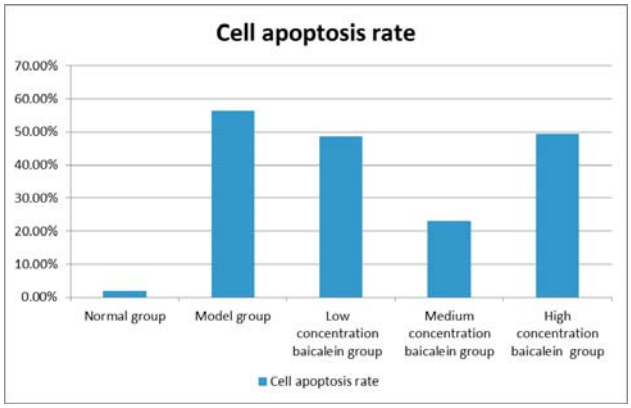

Fig. 2: Detection of apoptosis rate of cardiac myocytes

Effects of baicalein on myocardial infarction in isolated rat hearts

Compared with the normal group, the myocardial ischemia occurred in the model group, and the myocardial infarction rate was 63.14%±5.37. Compared with the model group, the myocardial ischemic state of the rats in the low, middle and high concentration groups of baicalein was improved, and the rate of myocardial infarction in the low and middle concentration groups was significantly decreased (P<0.05 or P<0.01), and there was no significant change in the high concentration group (P> 0.05), as shown in fig. 1.

Effects of baicalein on SOD activity, MDA content and GSH/GSSG ratio in myocardium

Compared with the normal group, the activity of SOD in the myocardial tissue of the rats in the model group decreased significantly, the content of MDA increased obviously, and the ratio of GSH/GSSG decreased significantly (P<0.01). Compared with the model group, the activity of SOD in the isolated heart myocardium of the rats with low and medium concentration of baicalein was significantly increased, and the content of MDA in the cardiac myocardium of the rats in the medium concentration group decreased significantly, and the ratio of GSH/GSSG increased significantly (P<0.01 or P< 0.05). There was no significant change in all indicators in high concentration group (P>0.05), and the results were shown in table 2.

Effects of baicalein on cardiomyocyte apoptosis in isolated rat hearts

Compared with the normal group, the apoptosis rate of isolated rat cardiac myocytes increased significantly in the model group (P<0.01). Compared with the model group, the apoptosis rate of cardiac myocytes in the isolated group of baicalein group was significantly lower (P<0.01). The apoptosis rate of cardiac myocytes in low and high concentration rats decreased slightly, but there was no significant difference (P>0.05), as shown in fig. 2.

DISCUSSION

The main reason of I/R damage is the production of reactive oxygen species (ROS). Under normal conditions, the concentration of ROS can be reduced by the antioxidant system (Kai *et al.,* 2015; Kargulewicz *et al.,* 2016). Antioxidant systems include antioxidant enzymes (such as SOD) and antioxidant molecules (such as GSH). However, when I/R occurs, oxidative stress will result in a large number of ROS, which is beyond the ability of antioxidant enzymes to clear ROS (Li *et al.,* 2015). Oxidative protection can reduce oxidative stress induced by ROS during reperfusion. Therefore, reducing oxidative stress can reduce I/R damage. Baicalein is generally recognized as having unique antioxidant activity (Mellotte *et al.,* 2015). The results showed that the concentration of baicalein could prevent I/R induced injury in myocardium, reduce the production of MDA and enhance the activity of SOD in myocardium, and increase the ratio of GSH/GSSG. Therefore, one of the mechanisms of baicalein's cardiac improvement is probably related to its antioxidant activity (Xuexiu *et al.,* 2015). However, the high concentration of baicalein preconditioning has no good regulation on oxidative stress, which may be because the high concentration of baicalein has the effect of promoting oxidation and promoting apoptosis.

I/R damage can lead to cardiac dysfunction and cardiomyocyte apoptosis. Significant myocardial dysfunction can be observed after I/R injury, which changes the release of some myocardial isozymes (such as CK, LDH, etc.), and induces myocardial infarction, and the apoptosis of cardiomyocytes is also observed. Some phenomena after I/R damage in this study are consistent with those in many other studies (Tsiaras *et al.,* 2016). The concentration of baicalein pretreatment could significantly reduce the release of CK and LDH induced

Table 1: LDH and CK activities detection results

Detection index	Group	10 min before stopping irrigation	Reperfusion 20 min	Reperfusion 40 min
LDH(U/L)	Normal group	16.42±4.17	16.23±4.06	16.10±5.52
	Model group	17.03±5.23	35.16±6.83	32.35±8.29
	Baicalein Low concentration group	16.58±3.02	41.95±3.84	36.17±5.28
	Baicalein medium concentration group	17.26±5.35	26.38±6.24	29.14±7.55
	Baicalein high concentration group	18.19±4.05	41.46±7.28	38.51±6.25
CK(U/L)	Normal group	25.31±7.28	23.15±6.24	22.13±4.28
	Model group	23.57±3.64	189.24±11.26	74.52±10.53
	Baicalein Low concentration group	24.13±5.04	206.83±15.06	93.27±9.42
	Baicalein medium concentration group	18.69±8.32	243.17±12.42	83.62±10.56
	Baicalein high concentration group	26.35±7.58	175.62±13.28	89.17±8.44

Table 2: Detection results of SOD activity, MDA content and GSH/GSSG ratio

Group	SOD (U/mg prot)	MDA (mol/kg prot)	GSH/GSSG
Normal group	9.65±0.74	215.58±17.20	36.42±1.37
Model group	2.46±0.53	410.17±14.15	22.36±0.61
Low concentration baicalein group	5.23±1.12	231.92±15.82	31.27±0.46
Medium concentration baicalein group	7.78±1.05	326.54±18.37	28.32±1.42
High concentration baicalein group	2.25±0.78	562.78±15.14	16.53±1.58

by I/R damage, decrease the myocardial infarction rate and decrease the rate of cardiomyocyte apoptosis. In the index of myocardial ischemia reperfusion, myocardial enzyme examination is of great value for the diagnosis of acute myocardial infarction (Roman *et al.,* 2011). The level of enzyme index is closely related to the amount of myocardial necrosis, and the level of myocardial injury is helpful to determine the degree of myocardial injury (Xuan 2015; Qin *et al.,* 2015). As the reperfusion injury and the permeability of the cell membrane increased, the LDH was leaked into the blood, the concentration of the serum increased, and the LDH had a strong cell damage, which could damage the cell membrane and numerous organelles, especially the damage to mitochondria was the most serious result (Yoshio *et al.,* 2013; Rosenthal *et al.,* 2015). Because the grain body is the only place to produce ATP, and it is the energy worker of the cell factory. Once ischemia and hypoxia, the ATP of cardiomyocytes decreases obviously, which further leads to a series of abnormalities and disorders in structure, function and metabolism (Zhu *et al.,* 2015). This study showed that the content of LDH in the serum of the model group was significantly higher than that of the model group ($P<0.05$). The content of LDH in the serum was significantly reduced after the use of Baicalein.

CONCLUSION

Baicalein is a kind of flavonoids. Long term use of flavonoids can reduce blood pressure by releasing physiological NO to keep the vascular endothelial function of hypertensive animals and reduce myocardial ischemia injury. To sum up, baicalein has a significant improvement in I/R damage, mainly in reducing myocardial infarction rate, reducing the release of myocardial isozyme LDH and CK, reducing the level of oxidative stress and reducing the apoptosis of cardiac myocytes. This suggests that the effect of baicalein on myocardium may be related to its antioxidative and anti apoptotic activity, but the role of baicalein in the cardiovascular system needs further test and clinical research.

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