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J Tradit Chin Med 2015 April 15; 35(2): 160-167
ISSN 0255-2922

EXPERIMENTAL STUDY

Effect of calycosin on left ventricular ejection fraction and angiogenesis in rat models with myocardial infarction

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Supported by the State Administration of Traditional Chinese Medicine Key Specialty Items; Shanghai Science and Technology Committee Project: Clinical Study of Intravascular Ultrasound and Fractional Flow Reserve of Coronary Artery Critical Evaluation Guidance of Interventional Treatment (No. 124119b1601); the Project of National Natural Science Foundation: the Effect of Ginkgolide B Drug Eluting Stents on Endothelialization and On P38mapk Signal (No. 81303145)

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Accepted: May 29, 2014

RESULTS: The construction of MI model resulted in a LVEF reduction of 50% compared with the sham-control. After 28 days, the LVEF value was 10% higher when calycosin (4 mg/kg) was administered compared with the DMSO group. The expression of VEGF and CD31 showed a dose-dependent manner when calycosin was administrated. The calycosin-treated (4 mg/kg) group displayed a two-fold increase in VEGF expression at both the mRNA and protein levels compared with the DMSO group. In addition, CD31 expression in the microvascular increased 1.5-fold in the 4 mg/kg calycosin-treated group.

CONCLUSION: Calycosin improved left ventricular ejection fraction in the MI rat models, induced VEGF expression in the ischaemic myocardium, increased CD31 expression and promoted angiogenesis.

Key words: Stroke volume; Angiogenesis inducing agents; Vascular endothelial growth factor A; Antigens, CD31; 7,3'-dihydroxy-4'-methoxyisoflavone

Abstract

OBJECTIVE: To evaluated the effect of calycosin on left ventricular ejection fraction and angiogenesis.

METHODS: Adult male Sprague-Dawley rats were randomly assigned into calycosin-treated groups (0.5, 1, 2, and 4 mg/kg qd), a dimethyl sulfoxide (DMSO), or a sham-operated control group. The myocardial ischaemia (MI) model was intraperitoneally administered calycosin for 28 days. The survival rates and left ventricular ejection fractions (LVEF) were compared between groups. The expression levels of vascular endothelial growth factor (VEGF) and cluster of differentiation 31 (CD31) in ischaemic myocardium were also measured and compared.

INTRODUCTION

Cardiovascular diseases (CVDs) is the leading cause of death worldwide. In 2011, approximately 230 million people were affected by CVD in China. Moreover, it is estimated that 9590 people die each day in China due to the diseases.[1] Myocardial ischaemia (MI) is one of the most common CVDs.[2] Myocardial ischaemia reperfusion (MIR) is recognized as one of the most suitble methods for MI treatment.[3] Studies indicate that this treatment leads to recanalisation, an increased survival

rate and improved left ventricular function.[4] Among several MIR methods, percutaneous coronary intervention (PCI) is recognized to be one of the fastest and most direct ways to save the sufferers from myocardium. Unfortunately, this method inherits unpredictable risks to cardiac function and, in some cases, even death, possibly because of the damage to the myocardium due to the infarcted vascular. Thus, promoting angiogenesis after MI is not only important to patients' recovery but is also crucial to the success of direct PCI.[5] Researchers have attempted to regenerate damaged myocardium by combining thrombolytic therapy, direct PCI and stem cell treatment together; however, the results have not been promising.[3,6,7] Thus, the rescue of impaired myocardium after surgery is still one obstacle in MI treatments, and promoting angiogenesis activity in MI patients remains an essential task for a successful treatment.

Calycosin is the major isoflavonoid in Huangqi (*Radix Astragali Mongolici*), synonyms: Astragalus membranaceus (Fisch.) Bunge and Astragalus membranaceus (Fisch.) Bunge var. mongholicus), a traditional Chinese herbal medicine, and is proven to promote the proliferation of the endodermis and angiogenesis.[8]) This isoflavonoid is proposed to be a new alternative drug for the treatment of cardiovascular diseases.[8,9] Calycosin exhibits similar effects as estrogen receptor modulators (SERMs) in zebrafish and human umbilical vein endothelial cells (HUVEC), which activate the MAPK signaling pathway and up-regulate vascular endothelial growth factor (VEGF).[9] The up-regulation of VEGF expression is closely related to angiogenesis,[10] which partially explains the mechanism of calycosin's effects in promoting endodermis proliferation. Although a mechanism for improving angiogenesis by using calycosin has been proposed, a detailed study involving an animal MI model is still required to demonstrate its clinical application potential further.

To demonstrate angiogenesis at the cellular level, cluster of differentiation 31 (CD31), also known as platelet endothelial cell adhesion molecule (PECAM)-1, was used in an immunohistochemical analysis. CD31 is a surface protein belonging to the immunoglobulin superfamily. This protein is involved in adhesion/signaling events and is expressed on the cell surface of endothelial cells (ECs).[11] CD31 is a well-known marker for ECs with vessel-forming activity because cells expressing the CD31 antigen exhibit increased endothelial repairing and revascularisation activities compared to CD31- cells. Thus, an increased number of CD31 + cells indicates a higher level of angiogenesis.[12]

This study aims to verify the potential of calycosin for treating MI. Left coronary artery ligation was used to construct rat MI models,[13,14] and the effects of calycosin on cardiac function were tested. VEGF and CD31 expression levels in the surviving myocardium were analyzed to elucidate further its mechanism in promoting angiogenesis in the models.

MATERIALS AND METHODS

Animals

The study was approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine. Seventy-two clean-grade male Sprague-Dawley rats of 6 weeks old weighing (220 ± 20) g, were used in the following experiments (Shanghai Songlian Experimental Animal Centre, approval number: SCXK Hu 2008-0016).

Apparatus and chemicals

Calycosin (purity > 98%, verified by high performance liquid chromatography; purchased from Sigma-Aldrich Co., St Louis, MO, USA) was dissolved in dimethyl sulphoxide (DMSO, from Sigma-Aldrich Co., St Louis, MO, USA) to make a 100 mM stock solution and stored at 4 ℃ until use. Huangqi (*Radix Astragali Mongolici*) used in this study was from the dried root of Astragalus propinquus. Pentobarbital, penicillin and heparin (150 U) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Animal echocardiography (Visualsonics, Toronto, Ontario, Canada) was used to assay the elution fractions. An animal physiological recorder (Chengdu instrument factory, Chengdu, China) was used in the intraoperative myocardial infarction model along with an electrocardiograph (ECG). A spectrophotometer biophotometer (Eppendorf, Hamburg, Germany), low-speed centrifuge 5702 (Eppendorf, Hamburg, Germany) and high-speed centrifuge 5804R (Eppendorf, Hamburg, Germany) were used in sample preparation. An RNA extraction kit (Omega, Doraville, CA, USA) was used to extract the total RNA. A murine anti-VEGF monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and fluorescence microscope (CKX41; Olympus, Tokyo, Japan) were used in the western blotting assay. A mouse anti-CD31 monoclonal antibody [mouse monoclonal (LCI-9) to CD31, Abcam, Cambridge, MA, USA], immunohistochemical washing buffer (Beyotime, Jiangsu, China), and immunohistochemical sealing buffer (Beyotime, Jiangsu, China) were used in immunohistochemical analysis.

Grouping and treatment

Seventy-two rats were randomly distributed into four calycosin-treated groups (0.5, 1, 2 and 4 mg/kg of calycosin were intraperitoneally injected after the construction of the model, n = 48), one DMSO group (vehicle control group) (DMSO was intraperitoneally injected after the construction of the model, n = 12) and one sham-operated control group (thorax was opened and closed without operation, n = 12). To ensure the absorbance of the calycosin in the rats, an intraperitoneal dose method was used. Calycosin was intraperitoneally injected after the construction of the model. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model.

Establishment of rat MI models

The rats were acclimated for one week and randomly assigned into each group. Before starting the surgery, the rats were anaesthetised by the intraperitoneal injection of 2.5% pentobarbital at a dose of 0.2 mL/100 g. For the calycosin-treated and DMSO groups, the heart was exposed via left thoracotomy at the fourth rib. The left anterior descending (LAD) artery was then permanently ligated. In the sham control group, the chest was opened after the LAD artery was located, and thread was then passed below the LAD artery without ligation. The chest was subsequently closed. Penicillin od 150 000 units was intramuscularly given to each rat for 3 days to prevent infection.

Left ventricular ejection fraction and Myocardial infarction size

The rats were anesthetized by intraperitoneal injection 2.5% pentobarbital at a dose of 0.2 mL/100 g and echocardiography was used at a frequency of 17.5 MHz to assay the left ventricular ejection fraction (LVEF). The following formula was used to calculate the LVEF: LVEF = ($LVDd^3$-$LVDs^3$)/$LVDd^3$, where LVDd is the left ventricular end-diastolic diameter, and LVDs is the left ventricular end-systolic diameter. The values for the 48th h and 28th day were calculated and compared in all groups.

The rats were anesthetized through the intraperitoneal injection of 2.5% pentobarbital and heparin (150 U) on the 28th day, and the hearts were then rapidly removed from each animal within the group. The white area of the myocardia was isolated, dried on pre-weighed filter paper and weighed. The weights of the necrotic myocardium for all 6 groups were analyzed and compared.

RNA extraction and real-time polymerase chain reaction (PCR)

The hearts extracted above were further utilised here. The surviving myocardium at the border of the infarction area was removed, and its total RNA was extracted using a RNA extraction kit according to the manufacturer's instructions. The primers for the real-time PCR of the mouse VEGF gene were designed by the Shanghai Bole biotechnology company: forward: 5¢-TGTACCTCCACCATGCCAAGT-3¢, and reverse: 5¢-TGGAAGATGTCCACCAGGGT-3¢. The reverse transcription was performed using a kit (Toyobo, Osaka, Japan), and the real-time PCR involved a 3-step method using β-actin as a housekeeping gene. The expression levels of the VEGF mRNA in all 6 groups were analyzed and compared.

Western blotting assay

The rats' hearts were extracted on the 28th day, and the surviving myocardium at the border of the infarction area was removed. Total extracts were prepared in ice-cold lysis buffer. The lysates were centrifuged at 12 000 ×*g* for 10 min (4 ℃). The total protein concentrations of the supernatants were determined using the Bradford method and bovine serum albumin as a standard. SDS-PAGE electrophoresis, blotting and the antibody reaction (mouse anti-VEGF, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were performed according to standard procedures and instructions. The chemiluminescent method was used for the detection and analysis. The VEGF expression results shown were normalized to the expression of β-actin.

Immunohistochemistry

The surviving cardiac muscle at the border of the infarction area of the extracted rat's heart was removed and sliced into 8 mm sections for immunohistochemistry. The sample was dyed in diluted CD31 (1∶500), and standard immunohistochemical methods were employed. The CD31-dyed microvasculature was detected and counted under a microscope (400 foldsmagnification).

Statistical analysis

The SPSS 17.0 (Chicago, IL, USA) was used to process tdata. Quantitative values are shown as the mean ± standard deviation ($\bar{x} \pm s$). Analysis of variancewas performed and followed by Dunnett's post hoc test. $P < 0.05$ was the significant level.

RESULTS

Establishment of the rat MI model

The details of the MI model construction are described in the Materials and Methods section. Of the preliminary experiments, 0.5 to 4 mg/kg of calycosin led to an angiogenic effect in the rat femoral artery ligation model (data not shown). Therefore, the same dosage range was used in the following experiments. After 48 h of surgery, two, three, two, three, two and two of the rats perished in the 0.5, 1, 2 and 4 mg/kg calycosin-treated, DMSO and control groups, respectively. The survival rates at 48 h were: 83.3, 75, 83.3, 75, 83.3 and 83.3%, respectively. The fatalities were predominantly caused by operational reasons, such as infection or pneumothorax. The average survival rate was 80.5% at 48 h. After 48 h, all of the remaining rats survived. ST segment elevation was observed in all the rats in the model groups by intraoperative monitoring on ECG, indicating the successful ligation of the LAD artery. No ST change was observed in the sham control group. In the model groups, there was no statistically significant difference in the size of the myocardial infarction area. The weights of the myocardial infarction areas were (0.174 ± 0.029), (0.171 ± 0.025), (0.169 ± 0.025), (0172 ± 0.023) and (0.169 ± 0.033) g for the 4 calycosin-treated (0.5, 1, 2 and 4 mg/kg of calycosin) and DMSO groups, respectively (Figure 1). These results indicate the successful construction of a MI model using rats, and this model was further used to evaluate the effects of calycosin.

Effect of calycosin on left ventricular ejection fraction

To elucidate the effects of calycosin on cardiac function in MI, echocardiography was used to assay the LVEF after 48 h in all six groups. Due to the MI, LVEF decreased significantly in the calycosin-treated and DMSO groups (Figure 1). During the first 48 h, more than 60% of the cardiac function was lost in all of the LAD artery ligated groups.

On the 28th day, LVEF was again assayed and calculated. Promisingly, cardiac function was improved, compared with the DMSO group, by treating the rats with 4 mg/kg of calycosin (P = 0.0419, Figure 2). Although a decrease in LVEF was observed in all groups, the reduction levels were relatively smaller for the calycosin-treated groups compared with the DMSO group (Figure 3). With the highest calycosin dose (4 mg/kg), the LVEF decreased by only 1.01% ± 0.71%, which was significantly lower compared to the DMSO group, which the LVEF decreased by more than 34% (P= 0.004).

Our results indicate that the effect of calycosin is dose-dependent and that the administration of a 4 mg/kg dose is efficacious in improving cardiac function in our MI model.

Effect of cal]ycosin on VEGF expression

Because angiogenesis is mediated by VEGF up-regulation, the expression level of VEGF was further assayed in the infarction-influenced myocardium. The surviving myocardium at the border of the infarction area was removed from the rats' hearts on the 28th day, and its total RNA was extracted. A 3-step method was used along with real-time PCR to analyze the different expression levels of VEGF mRNA for each group. As shown in Figure 3, compared with the sham control group, VEGF expression was increased in the DMSO group, indicating the self-rescue of the myocardium in response to MI. As expected, even higher expression levels of VEGF were observed in the calycosin-treated groups (Figure 4). Thus, it is clear that the administration of calycosin enhanced the angiogenesis signal.

Consistent with the improvement in cardiac function,

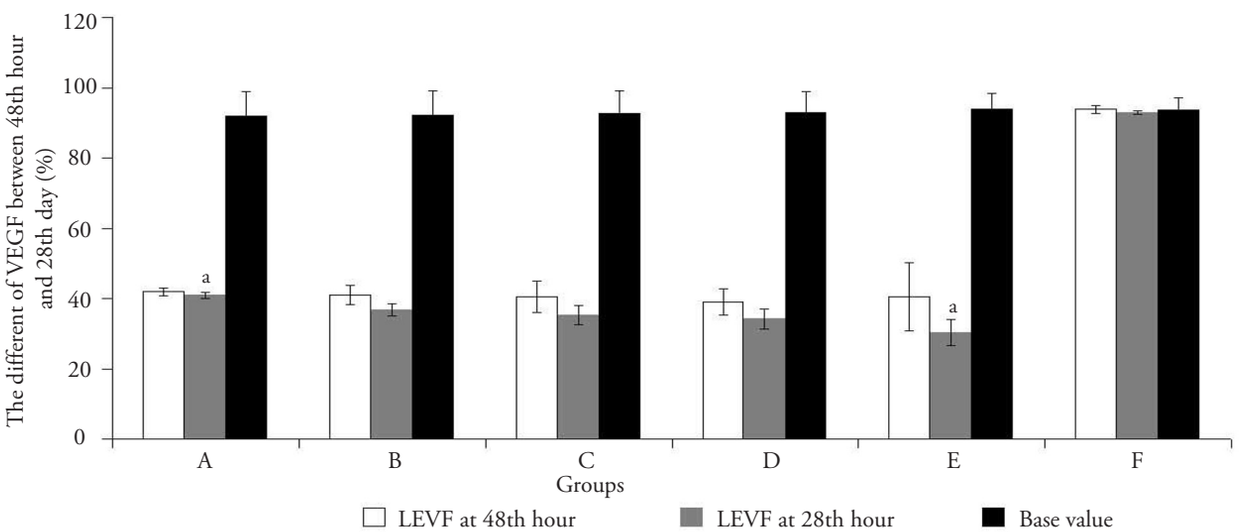

Figure 1 LVEF in calycosin, DMSO and sham-control groups at 48th h and 28th day

A-D: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; E: treated with DMSO; F: sham-control. Four different doses of calycosin were intraperitoneally injected after the construction of the myocardial ischaemia model. LVEF: left ventricular ejection fractions; DMSO: dimethyl sulfoxide. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model. Data were expressed as mean ± standard deviation (n = 9). Difference with $^aP < 0.05$ was considered as statistically significant and were indicated with an asterisk.

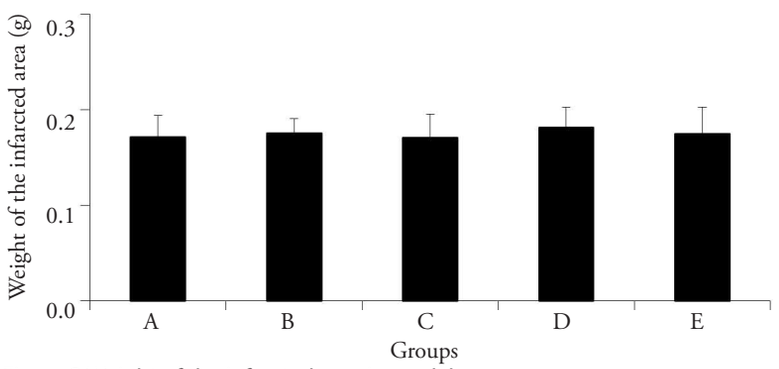

Figure 2 Weight of the infarcted area in model groups

A-D: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; E: treated with DMSO. DMSO: dimethyl sulfoxide. Data were expressed as mean ± standard deviation (n = 9). Four different doses of calycosin were intraperitoneally injected after the construction of the myocardial ischaemiamodel. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model.

VEGF was induced by calycosin in a dose-dependent manner. Compared with the DMSO group, a twofold increase in VEGF expression was achieved by administering 4 mg/kg of calycosin. A statistically significant higher level of vegf expression with respect to the DMSO group was observed with a calycosin dose as low as 1 mg/kg (P = 0.01).

The expression level of β-actin was stable in all samples thus the expression level of the VEGF protein is expressed as the fold-change relative to the β-actin expression level in the following results (Figure 5 A and B). A nearly 3-fold increase in VEGF protein expression was observed in the DMSO group compared with the sham control, and the expression level of the VEGF protein was further increased in the entire calycosin-treated group. Similar to the mRNA expression results, the administration of 4 mg/kg of calycosin resulted in the largest induction. VEGF protein expression increased by more than 2-fold compared with the DMSO group at this dose, verifying the enhancement of the angiogenesis signaling pathway by adding 4 mg/kg calycosin. From the mRNA and protein expression results for VEGF, calycosin was demonstrated to induce an angiogenesis signal in the rat MI model. To investigate whether this induction functionally promotes angiogenesis, the cellular response was further analyzed.

Effect of calycosin on CD31 expression

To evaluate angiogenesis in the myocardium, the number of cells expressing CD31 was analyzed by immunohistochemistry. CD31 is a specific antibody in the vascular endodermis. Therefore, its expression level can be used to indicate angiogenesis in the ischaemic myocardium vascular. The surviving myocardium was dyed using standard immunohistochemical methods, and the CD31-dyed microvasculature was counted (Figure 6). It was observed that the number of CD31+ microvascular cells doubled in the 4 mg/kg calycosin-treated group compared with the control, indicating angiogenic promotion in the surviving myocardium (Figure 7). This result coincides with the elevated ability for myocardial regeneration in the calycosin-treated groups. Thus, it is quite possible that due to its promotion of angiogenesis in the ischaemic myocardium, calycosin significantly improved the LVEF in the MI model.

Surprisingly, there was no increase in the number of CD31 cells in the DMSO group compared with the control in spite of its 2-fold higher mRNA level and 3-fold higher protein level for VEGF. This fact explains the continuously decreased cardiac function of the

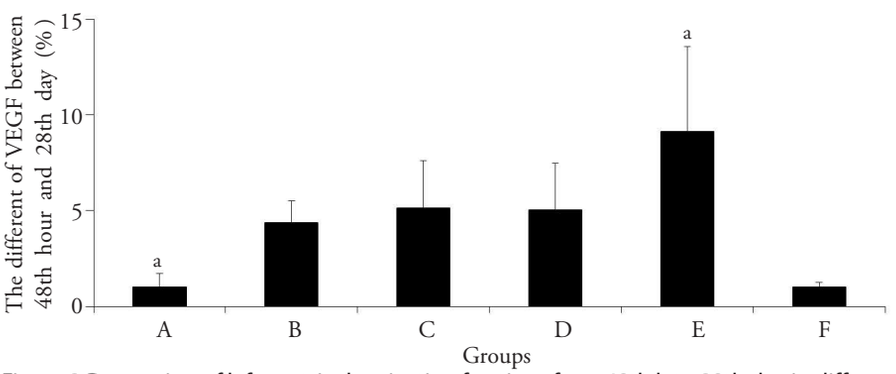

Figure 3 Decreasing of left ventricular ejection fractions from 48th h to 28th day in different groups

A-D: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; E: treated with DMSO; F: sham-control. Four different doses of calycosin were intraperitoneally injected after the construction of the myocardial ischaemia model. DMSO: dimethyl sulfoxide. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model. Data were expressed as mean ± standard deviation (n = 9). Difference with $^aP < 0.05$ was considered as statistically significant and were indicated with an asterisk.

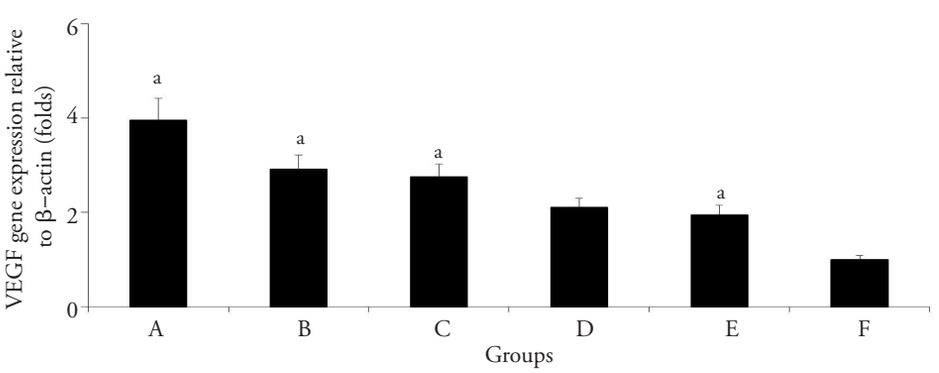

Figure 4 Transcriptional expression of vascular endothelial growth factorin survived myocardium in different groups

A-D: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; E: treated with DMSO; F: sham-control. Four different doses of calycosin were intraperitoneally injected after the construction of the myocardial ischaemia model. DMSO: dimethyl sulfoxide. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model. Data were expressed as mean ± standard deviation (n = 6). Difference with $^aP < 0.05$ was considered as statistically significant and were indicated with an asterisk.

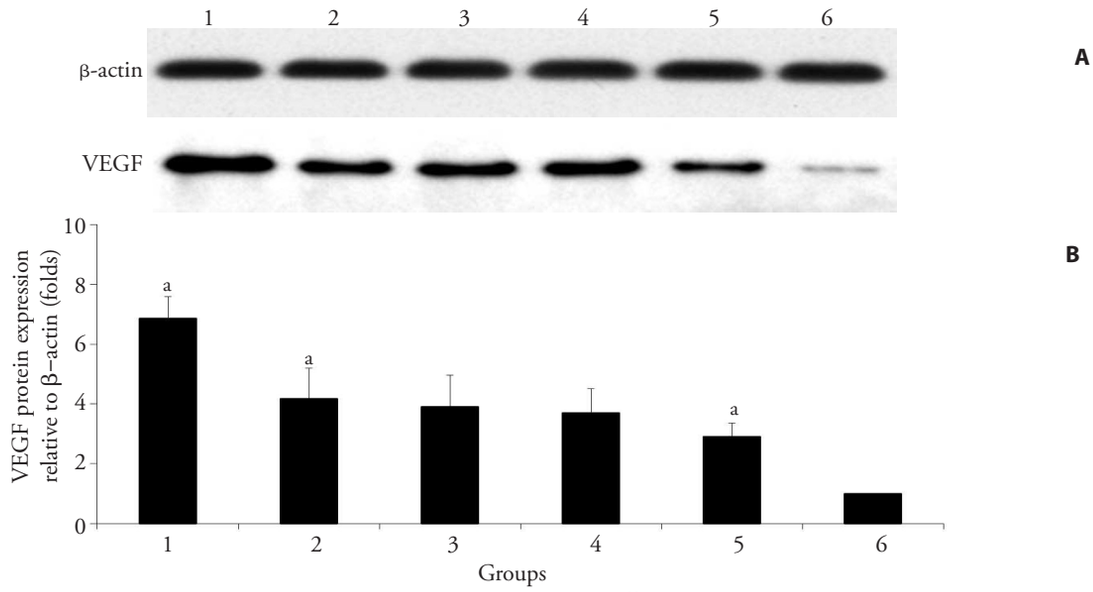

Figure 5 VEGF protein expression in survived myocardium in different groups

A: expression of β-actin in different groups; B: the relative expression level of VEGF. 1 to 4: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; 5: treated with DMSO; 6: sham-control. DMSO: dimethyl sulfoxide; VEGF: vascular endothelial growth factor. Four different doses of calycosin were intraperitoneally injected after the construction of the MI model. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model. Data were expressed as mean ± standard deviation (n = 6). Difference with $^{a}P < 0.05$ was considered as statistically significant and were indicated with an asterisk.

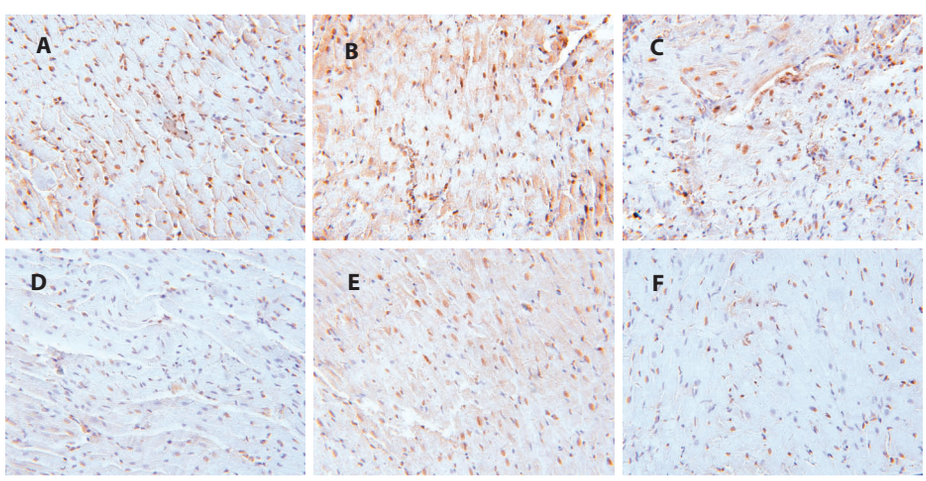

Figure 6 CD31 expression in different groups (Immunohistochemical staining, ×400)

A to D: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; E: treated with DMSO; F: sham-control. DMSO: dimethyl sulfoxide. The sample was dyed in diluted CD31 (1∶500), and standard immunohistochemical methods were employed. The CD31-dyed microvasculature was detected and counted under a microscope. The purple dots indicated nucleus of the micro-vascular and the brown dots indicated the CD31+ micro-vascular. Four different doses of calycosin were intraperitoneally injected after the construction of the myocardial ischaemia (MI) model. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model.

DMSO group and further demonstrates the necessity for calycosin supplementation during MI treatment.

DISCUSSION

In this study, the results of an echocardiography assay indicate that the reduction of LVEF was prevented in the calycosin-treated groups in the MI model. When the dose increased to 4 mg/kg, cardiac function did not further reduce from the 2nd to 28th day, suggesting its effects in improving cardiac function during MI. As a result, compared with the DMSO group, the LVEF value was 10% higher when 4 mg/kg of calycosin was administered, which is better than current reported drugs in MI models.

Calycosin is a phytoestrogen, and its function is reported to involve the estrogen receptor and mitogen-activated protein kinase activation/modulation.[9] Estrogen reduction results in risks for several diseases, especially CVDs in postmenopausal women. However, estrogen replacement therapy increases the risk of breast cancer, stroke and thrombus-related diseases, as indicated by recent epidemiology studies.[15,16] These results have prompted scientists to identify suitable phytoestrogens to treat diseases that result from estrogen reduction. In this study, calycosin exhibited functions similar to

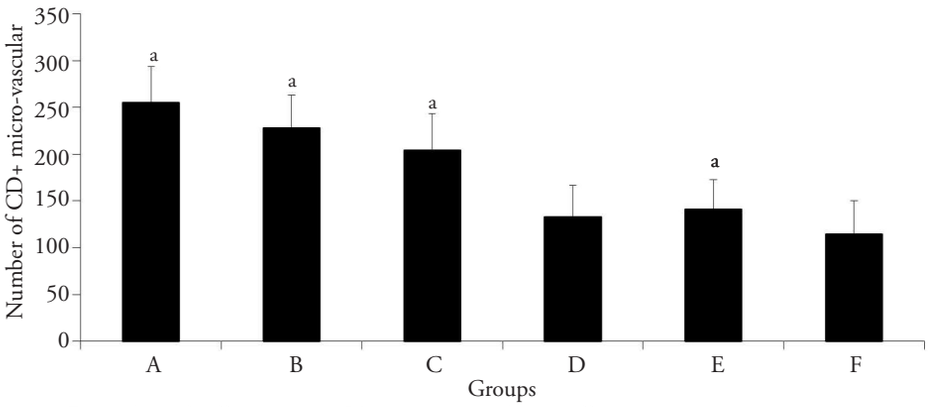

Figure 7 Number of differentiation 31+ micro-vascular in different groups

Four different doses of calycosin were intraperitoneally injected after the construction of the myocardial ischaemia (MI) model. The rats were intervened by the daily administration of calycosin for 28 days after the construction of the model. A to D: treated with 4, 2, 1 and 0.5 mg/kg of calycosin, respectively; E: treated with DMSO; F: sham-control. DMSO: dimethyl sulfoxide. Data were expressed as mean ± standard deviation (n = 6). Difference with $^{a}P < 0.05$ was considered as statistically significant and were indicated with an asterisk.

those of estrogen, which substantially increased VEGF expression at both the transcription and translation levels in a dose-dependent manner. A similar induction of VEGF expression by calycosin administration has also been observed in zebra fish and HUVEC. It has been demonstrated that calycosin can interact with estrogen receptors and activate the mitogen-activated protein kinase (MAPK) signaling pathway. In the MI model, the vegf expression in the myocardium was enhanced by the administration of calycosin, suggesting that a similar signal transduction event mediated the calycosin-led effects. Although molecular responses to calycosin were observed at doses as low as 1 mg/kg, only 4 mg/kg of calycosin resulted in an obvious improvement in cardiac function and angiogenesis.

This dose-dependent calycosin effect is very informative for understanding myocardial regeneration in a MI model. During MI, cells spontaneously respond to an infarction at the molecular level. This phenomenon has been reported as ischaemia-induced angiogenesis.[10] Both the transcriptional and translational levels of VEGF drastically increased in the myocardium due to the MI-induced ischaemia. However, the substantially increased expression of VEGF did not yield a detectable benefit on either cardiac function or the angiogenesis of the myocardial vascular. This phenomenon indicates that angiogenesis activation requires the signal strength to reach an excessively high level. Although several drugs can induce the angiogenesis signal, the strength is still below the threshold, resulting in little improvement in cardiac function. Because functional angiogenesis is difficult to activate without the proper intervention, a high risk of myocardium death is unavoidable after MI treatment.

A similar nonfunctional induction of VEGF was also observed when a lower dose of calycosin was administered. The injection of 0.5 mg/kg of calycosin enhanced VEGF expression compared to the control; however, an improvement in angiogenesis was only observed when above 1 mg/kg of calycosin was administered. Moreover, improved cardiac function was only observed when 4 mg/kg of calycosin was used. At this dose, VEGF expression was increased by 4- and 7-fold at the mRNA and protein levels, respectively. Based on this result, the injection of 4 mg/kg of calycosin is proposed to be a suitable dose to activate angiogenesis and to improve cardiac function in MI. In as short a time as 28 days, a significant improvement in cardiac function and angiogenesis was observed compared to the DMSO group. Because of the activation of angiogenesis, this improvement is expected to be even greater in the long term; however, such an observation is still pending.

In conclusion,calycosin improves the cardiac function of MI rats through its induction of VEGF expression in the ischaemic myocardium and its acceleration of angiogenesis. In addition, the CD31 + cell number is also increased by calycosin treatment, which further improves the reconstruction of the infarcted vasculature. The above results demonstrate that calycosin is a potential candidate for myocardial infarction treatment.

REFERENCES

1 **Hu S**, Kong L. Report on cardiovascular deceases in China 2011. Beijing: Encyclopedia of China Publishing House, 2012: 1-3.

2 **Patel V**, Upaganlawar A, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced MI in rats, a biochemical, electrocardiographic and histoarchitectural evaluation. Eur J Pharmacol 2010; 644 (1): 160-168.

3 **Danchin N**, Coste P, Ferrières J, et al. Comparison of thrombolysis followed by broad use of percutaneous coronary intervention with primary percutaneous coronary intervention for ST-segment-elevation acute MI: Data from the French registry on acute ST-elevation MI (FAST-MI). Circulation 2008; 118(3): 268-276.

4 **Ryan TJ**, Antman EM, Brooks NH, et al. 1999 update: ACC/AHA guidelines for the management of patients with acute MI: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Acute MI). J Am Coll Cardiol 1999; 34(3): 890-911.

5 **Holash J**, Wiegand SJ, Yancopoulos GD. New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene 1999; 18(38): 5356-5362.

6 **Miettinen JA**, Ylitalo KV, Niemelä M, et al. Left ventricular functional recovery after intracoronary injection of autologous bone marrow-derived stem cells in patients with acute MI: A dose-response pilot study. Int J Cardiol 2012; 154(3): 354-356.

7 **Mansour S**, Roy DC, Bouchard V, et al. One-year safety analysis of the COMPARE-AMI trial: Comparison of intracoronary injection of CD133 bone marrow stem cells to placebo in patients after acute MI and left ventricular dysfunction. Bone Marrow Res 2011; 2011(1): 1-6.

8 **Li S**, Lou S, Lei BU, et al. Transcriptional profiling of angiogenesis activities of calycosin in zebrafish. Mol Biosyst 2011; 7(11): 3112-3121.

9 **Tang JY**, Li S, Li ZH, et al. Calycosin promotes angiogenesis involving estrogen receptor and mitogen-activated protein kinase (MAPK) signaling pathway in zebrafish and HUVEC. PLoS ONE 2010; 5(7): e11822.

10 **Zaitone SA**, Abo-Gresha NM. Rosuvastatin promotes angiogenesis and reverses isoproterenol-induced acute MI in rats: Role of iNOS and VEGF. Eur J Pharmacol 2012; 691 (1): 134-142.

11 **Kim SW**, Kim H, Yoon Y. Advances in bone marrow-derived cell therapy: CD31-expressing cells as next generation cardiovascular cell therapy. Regen Med 2011; 6(3): 335-349.

12 **Weil BR**, Kusher EJ, Diehl KJ, Greiner JJ, Stauffer BL, DeSouza CA. CD31 + T cells, endothelial function and cardiovascular risk. Heart Lung Circ 2011; 20(10): 659-662.

13 **Ou L**, Li W, Zhang Y, et al. Intracardiac injection of matrigel induces stem cell recruitment and improves cardiac functions in a rat MI model. J Cell Mol Med 2011; 15(6): 1301-1318.

14 **Shao MJ**, Wang SR, Zhao MJ, et al. The effects of velvet antler of deer on cardiac functions of rats with heart failure following MI. Evid Based Complement Alternat Med 2012; 2012(1): 1-5.

15 **Wren BG**. The benefits of oestrogen following menopause: Why hormone replacement therapy should be offered to postmenopausal women. Med J 2009; 190(6): 321-325.

16 **Conner P**, Lundstrom E, von Schoultz B. Breast cancer and hormonal therapy. Clin Obstet Gynecol 2008; 51(3): 592-606.