Atorvastatin combined with routine therapy on HIF-1, VEGF concentration and cardiac function in rats with acute myocardial infarction

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Abstract. Effect of atorvastatin combined with routine therapy on the expression of hypoxia inducible factor (HIF-1) and vascular endothelial growth factor (VEGF) in rats with acute myocardial infarction (AMI) and its therapeutic effect were investigated. The rat models of acute myocardial infarction were established and divided into routine therapy, study, model, single drug and control group according to the treatment plan, with 10 cases in each group. Enzyme-linked immunosorbent assay (ELISA) was used to detect the concentration of HIF-1 and VEGF in serum of rats before treatment (T0), and 3 days (T1), 5 days (T2) and 7 days (T3) after treatment, and the cardiac function was measured at the same time. The concentrations of HIF-1 and VEGF in serum of the study group and the routine therapy group after treatment were significantly higher than those before treatment. The concentrations of HIF-1 and VEGF in serum of model group at T0 were significantly lower than those at T3 ($P<0.05$). After treatment, the concentrations of HIF-1 and VEGF in serum of the study group were significantly higher than those of the routine therapy, the model and the control group ($P<0.05$). One week after administration, there were significant differences in the left ventricular function among the five groups ($P<0.001$). The left ventricular function in the study group was better than that in the routine therapy, model and control group. The levels of HIF-1 and VEGF in the serum of rats with myocardial infarction were negatively correlated with LVIDs and LVIDd, and positively correlated with LVEF% and LVFS%. In conclusion, atorvastatin combined with routine therapy can better reduce serum HIF-1 and VEGF levels and improve the left ventricular function in rats than routine therapy.

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Introduction

Acute myocardial infarction refers to myocardial necrosis caused by acute, persistent ischemia and hypoxia (coronary artery dysfunction) (1). This disease has a rapid onset and a high mortality rate causing great harm to the health of patients (2). There have been many advances in the treatment of myocardial infarction. Rapid thrombus aspiration or PCI is the first choice in patients with acute myocardial infarction, and the routine treatment is anticoagulation and thrombolysis (3,4). However, it has been found in clinic that even if infarction-related blood vessels were opened at first occurence, not all cardiomyocytes could obtain effective blood perfusion (5), which led to poor prognosis. When acute myocardial infarction occurs, how to reduce thrombosis and increase myocardial blood supply is a hot research topic at present (6).

Atorvastatin is a new generation of HMG-CoA reductase inhibitor, which reduces the content of blood lipid in blood vessels by inhibiting cholesterol synthesis in hepatocytes. It has pharmacological effects of anti-inflammation, lipid regulation and improvement of ventricular function (7), and can improve ventricular ejection fraction and weaken myocardial remodeling in patients with ischemia-induced heart failure (8). Recently, some studies have reported that high-dose of atorvastatin (40 mg) pretreatment can improve the clinical outcome of patients with myocardial infarction undergoing emergency percutaneous coronary intervention (9,10).

Hypoxia inducible factor (HIF) is a transcription factor that responds to the reduction or hypoxia of available oxygen in the cell environment (11). The HIF-1 signal cascade reaction mediates the effects of hypoxia on the cells, which typically allow the cells to differentiate continuously, promote the formation of blood vessels, and are important for the formation of a vascular system in an embryo and a tumor. Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulate blood vessels and is part of the system of restoring tissue oxygen supply. The normal function of VEGF is to produce new blood vessels and collateral circulation during embryonic development, which plays an indirect role in improving neuronal vascular perfusion (12). At present, it has been reported that the concentrations of VEGF and HIF-1α may be related to myocardial remodeling and angiogenesis (13). It also has been reported that HIF-1 may be an ischemic response medium and has the potential diagnostic effect of HIF-1 tissue

table as a marker of ischemia (14), which can effectively reflect the process of acute myocardial infarction to a certain extent. In this study, the effect of atorvastatin combined with routine therapy on the concentrations of HIF-1 and VEGF in rats with acute myocardial infarction was explored, and the therapeutic effect of atorvastatin combined with routine therapy was investigated, so as to provide reference for clinical diagnosis and treatment of acute myocardial infarction.

Materials and methods

General materials. Fifty male Wistar rats were purchased from the Experimental Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China), and were randomly divided into 5 groups (n=10). The rats were 2 months old and weighed 200-220 g. Four groups were modeled for myocardial infarction, while one group (the control group) was not treated. From the four successful modeled groups, one group of rats was randomly selected as the routine therapy group to receive intravenous infusion of nitroglycerin (0.3 mg/kg) (Shandong Miyoshi Medicine Co., Ltd.; H13022503) and aspirin (20 mg/kg/d) (CR Double-Crane; H11022441). Another group of rats (the study group) was treated with intravenous nitroglycerin and aspirin combined with atorvastatin (20 mg/kg/d) (Pfizer; H20051408). One group (the single drug group) was only treated with atorvastatin (20 mg/kg/d). The last group (the model group) did not receive any treatment. Rats were kept in cages with controlled temperature, light cycles and humidity (23˚C, 12/12 light cycles, 55±10%) and with free access to water.

Empirical method

Establishment of rat model of acute myocardial infarction. The rat model of acute myocardial infarction (AMI) was established. The rats were anesthetized by intraperitoneal injection, and then endotracheal intubation connected with ventilator was performed to assist respiration. Multi-channel physiological signal acquisition and processing system were connected to record the electrocardiogram (ECG). The chest was opened, and the heart was exposed. The pericardium was cut, and the left anterior descending branch of the coronary artery was ligated by non-invasive suture. The thoracic cavity was closed layer by layer, and routinely disinfected to prevent infection. The rats were fed in a single cage. The standard lead II on the multi-channel physiological recorder was observed. If the ST-segment elevation and/or T-wave elevation or decrease was observed, and the muscle tissue around the rat appeared pale or dark, the modeling was successful.

Medication. In the control group and the model group, the mice were given intragastric administration of the same amount of saline once a day for 7 days. Twenty-four hours after modeling, nitroglycerin (0.3 mg/kg) (Shandong Miyoshi Medicine Co., Ltd.; H13022503) and aspirin (20 mg/kg/d) (CR Double-Crane; H11022441) were given intravenously to the routine therapy group. The study group was given atovastatin (20 mg/kg/d) (Pfizer; H20051408) on the basis of routine treatment, once a day, intragastrically for 7 days. The single drug group was only treated with atorvastatin. All experimental steps were approved by the Animal Ethics Committee of Zibo Central Hospital (Zibo, China).

Determination of HIF-1, VEGF content in serum of rats in each group. The venous blood of the rats was taken before treatment (T0), and 3 days (T1), 5 days (T2), and 7 days (T3) after treatment. The blood sample was centrifuged. The upper serum was obtained after centrifugation (2,000 x g, at 4˚C for 15 min). The levels of HIF-1 (Shanghai Guangrui Biotechnology Co., Ltd.; rat elisa389) and VEGF (Shanghai Guangrui Biotechnology Co., Ltd.; rat elisa179) in the serum of the rats were detected by ELISA. The above steps were performed strictly according to the instructions of the kit.

Examination of left ventricular function in each group. After anesthesia with 2% xylazine (5 mg/kg) and 5% ketamine (35 mg/kg) (15) residual hair in the left chest was removed by hair removal cream and skin preparation knife after anaesthesia administration. Echocardiography was used to determine cardiac function. The left ventricular internal diameter at systole (LVIDs), left ventricular diastolic diameter (LVIDd), left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) were measured.

Observation index. The concentrations of HIF-1 and VEGF in the serum of rats in each group; comparison of the effects of left ventricular function in each group of rats; correlation between left ventricular function and HIF-1 and VEGF in rats.

Statistical analysis. The SPSS 24.0 (IBM Corp., Armonk, NY, USA) software was used to statistically calculate all experimental results. All graphics were plotted by GraphPad 8 (Soft Head Inc.) and the results were checked twice. The measurement data are presented as the mean ± standard deviation. One factor variance analysis and LSD (Least-Significant Difference) post test were used for the comparison among groups. Variance analysis of repeated measurement and bonferroni post-test were used for the comparison of data among multiple time-points. Pearson's correlation coefficient was used for the correlation analysis. Statistical significance was set at $P<0.05$.

Results

Modeling results. Forty rats were successfully modeled with acute myocardial infarction (AMI). The success rate of modeling was 100%.

Concentration of HIF-1 and VEGF in rat serum at T0, T1, T2 and T3. There were significant differences in HIF-1 at T0, T1, T2 and T3 among the groups ($P<0.001$), and the control group was the lowest at each time-point. There was a significant difference in HIF-1 among multiple time-points within the group ($P<0.001$). There was no significant difference in HIF-1 at T0, T1, T2 and T3 in the control group ($P>0.05$). In the model group, routine therapy group, single drug group and study group, HIF-1 at T0 was the lowest, T1 was higher than T0, and T3 was the highest. There were significant differences in VEGF at T0, T1, T2 and T3 among the groups ($P<0.001$); the control group was the lowest, and the study group was the highest at each time-point. There was a significant difference in VEGF among multiple time-points within the group ($P<0.001$) (Table I). There was no significant difference in VEGF at T0, T1, T2 and T3 in the

Table I. Comparison of HIF-1 concentrations at different time-points in the four groups (ng/ml, n=10).

Time-point	Control group	Model group	Routine therapy group	Single drug group	Study group	F value	P-value
T0	0.28±0.09	1.12±0.07[a]	1.11±0.06[a]	1.12±0.05[a,e]	1.13±0.11[a]	226.21	<0.001
T1	0.26±0.17	1.36±0.11[a,e]	1.66±0.12[a,b,e]	1.67±0.15[a,b,e]	1.76±0.13[a-e]	205.165	<0.001
T2	0.27±0.32	1.54±0.14[a,e,f]	1.79±0.15[a,b,e,f]	1.76±0.16[a,b,e,f]	1.96±0.16[a-f]	119.513	<0.001
T3	0.30±0.17	1.73±0.09[a,e-g]	2.25±0.24[a,b,e-g]	2.23±0.26[a,b,e-g]	2.69±0.23[a-g]	199.124	<0.001
F value	0.069	60.51	89.74	99.79	153.7		
P-value	0.976	<0.001	<0.001	<0.001	<0.001		

[a]P<0.05, compared with the control group at the same time-point; [b]P<0.05, compared with the model group at the same time-point; [c]P<0.05, compared with the routine therapy group at the same time-point; [d]P<0.05, compared with the single drug group at the same time-point; [e]P<0.05, compared with HIF-1 at T0 within the group; [f]P<0.05, compared with HIF-1 at T1 within the group; [g]P<0.05, compared with HIF-1 at T2 within the group. HIF-1, hypoxia inducible factor.

Table II. Comparison of VEGF concentrations at different time-points in the four groups (ng/ml, n=10).

Time-point	Control group	Model group	Routine therapy group	Single drug group	Study group	F value	P-value
T0	0.09±0.10	0.31±0.12[a]	0.26±0.05[a,b]	0.25±0.06[a,e]	0.27±0.12[a-c]	7.996	<0.001
T1	0.09±0.16	0.37±0.07[a,e]	0.54±0.14[a,b,e]	0.55±0.12[a,b,e]	0.66±0.12[a-e]	74.153	<0.001
T2	0.09±0.17	0.51±0.08[a,e,f]	0.69±0.09[a,b,e,f]	0.68±0.08[a,b,e,f]	0.97±0.13[a-f]	100.314	<0.001
T3	0.09±0.12	0.62±0.14[a,e-g]	0.96±0.27[a,b,e-g]	0.95±0.28[a,b,e-g]	1.25±0.23[a-g]	41.134	<0.001
F value	1.000	17.218	33.142	32.912	71.843		
P-value	0.404	<0.001	<0.001	<0.001	<0.001		

[a]P<0.05, compared with the control group at the same time-point; [b]P<0.05, compared with the model group at the same time-point; [c]P<0.05, compared with the routine therapy group at the same time-point; [d]P<0.05, compared with the single drug group; [e]P<0.05, compared with VEGF at T0 within the group; [f]P<0.05, compared with VEGF at T1 within the group; [g]P<0.05, compared with VEGF at T2 within the group. VEGF, vascular endothelial growth factor.

Table III. Comparison of left ventricular function in each group of rats.

Index	Control group	Model group	Routine therapy group	Single drug group	Study group	F value	P-value
LVIDs (mm)	4.47±0.52	8.21±2.18[a]	6.59±2.01[a,b]	6.58±2.14[a,b]	5.28±1.38[a-d]	9.745	<0.001
LVIDd (mm)	6.07±0.47	10.37±1.72[a]	9.82±1.38[a,b]	9.85±1.29[a,b]	8.26±1.48[a-d]	20.413	<0.001
LVEF%	70.50±4.61	25.62±3.73[a]	55.82±3.04[a,b]	54.96±3.14[a,b]	63.18±1.02[a-d]	348.1	<0.001
LVFS%	44.93±4.08	23.63±4.02[a]	31.38±3.92[a,b]	31.54±4.03[a,b]	39.74±3.07[a-d]	60.982	<0.001

[a]P<0.05, compared with the control group; [b]P<0.05, compared with the model group; [c]P<0.05, compared with the routine therapy group; [d]P<0.05, compared with the single drug group.

control group (P>0.05). In the model group, routine therapy group, single drug group and study group, VEGF at T0 was the lowest, T1 was higher than T0 and T3 was the highest (Table II).

Comparison of left ventricular function in each group one week after administration. One week after administration, there was significant difference in left ventricular function among the five groups (P<0.001). The LVIDs and LVIDd of the model group, the routine therapy group, single drug group and the study group were higher than those of the control group, and the LVIDs and LVIDd were lowest among them. The LVEF and LVFS of the model group, the routine therapy group, single drug group and the study group were lower than those of the control group (P<0.001), and the LVEF and LVFS were the highest among them (P<0.001) (Table III).

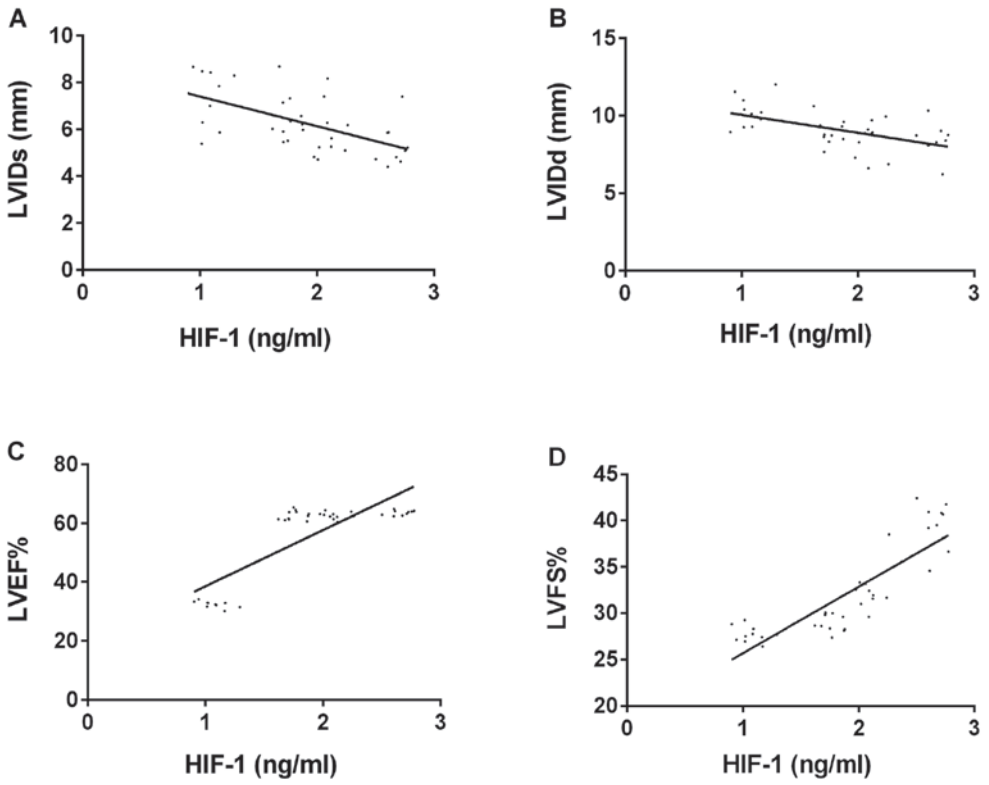

Figure 1. Correlation analysis between LVIDs, LVIDd, LVEF, LVFS and HIF-1 levels. (A) Pearson's correlation coefficient analysis showed that HIF-1 level was negatively correlated with LVIDs (r=-0.580, P=0.001). (B) Pearson's correlation coefficient analysis showed that HIF-1 level was negatively correlated with LVIDd (r=-0.548, P=0.001). (C) Pearson's correlation coefficient analysis showed that HIF-1 level was positively correlated with LVEF (r=0.822, P=0.001). (D) Pearson's correlation coefficient analysis showed that HIF-1 level was positively correlated with LVFS (r=0.853, P=0.001). HIF-1, hypoxia inducible factor-1; VEGF, vascular endothelial growth factor; LVIDs, left ventricular internal diameter at systole; LVIDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

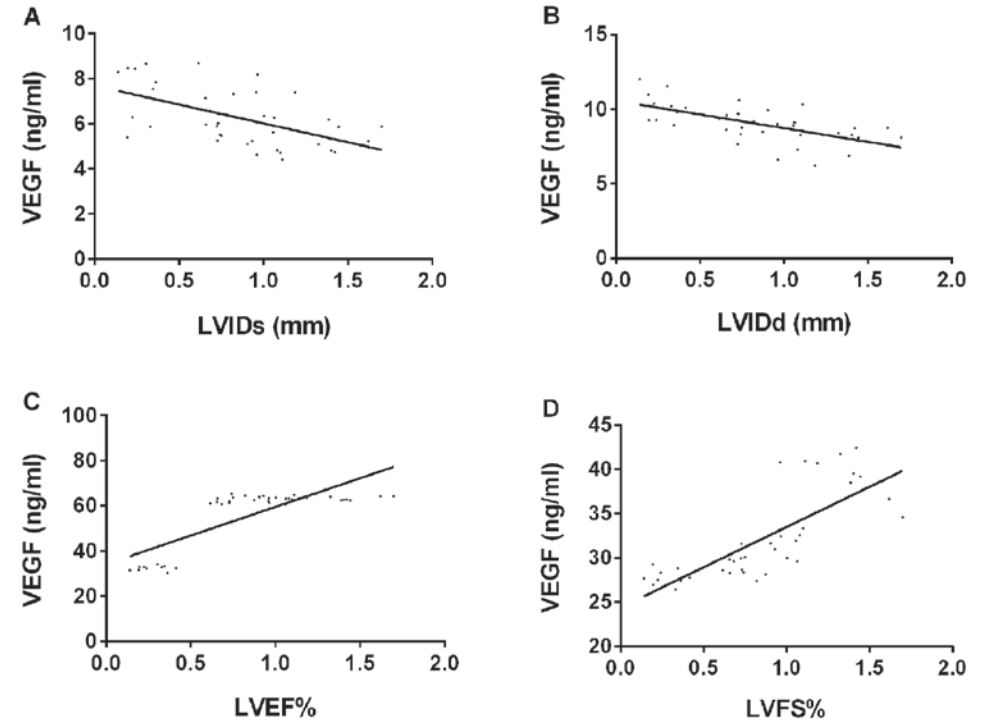

Figure 2. Correlation analysis between LVIDs, LVIDd, LVEF, LVFS and VEGF levels. (A) Pearson's correlation coefficient analysis showed that VEGF level was negatively correlated with LVIDs (r=-0.555, P=0.044). (B) Pearson's correlation coefficient analysis showed that VEGF level was negatively correlated with LVIDd (r=-0.613, P=0.001). (C) Pearson's correlation coefficient analysis showed that VEGF level was positively correlated with LVED (r=0.791, P=0.001). (D) Pearson's correlation coefficient analysis showed that VEGF level was positively correlated with LVFS (r=0.783, P=0.001). VEGF, vascular endothelial growth factor; LVIDs, left ventricular internal diameter at systole; LVIDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

Analysis of the correlation between HIF-1, VEGF and left ventricular function. The level of HIF-1 in the serum of rats with myocardial infarction was negatively correlated with LVIDs (r=-0.580, P=0.001) and LVIDd (r=-0.548, P=0.001), but was positively correlated with LVEF (r=0.822, P=0.001) and LVFS (r=0.853, P=0.001) (Fig. 1). VEGF level was negatively correlated with LVIDs (r=-0.555, P=0.044) and LVIDd (r=-0.613, P=0.001), but was positively correlated with LVEF (r=0.791, P=0.001) and LVFS (r=0.783, P=0.001) (Fig. 2).

Discussion

Acute myocardial infarction, one of the diseases threatening human life and health, not only has a very rapid onset, but also a very poor prognosis (16). In recent years, the incidence of myocardial infarction has shown an upward trend. The number of deaths caused by cardiovascular disease is higher than that caused by cancer or any other disease (17). At present, the focus of the treatment of acute myocardial infarction is how to improve the survival rate of patients (18), and atorvastatin has been proved to have a good effect in the treatment of acute myocardial infarction (19). The effects of atorvastatin combined with conventional treatment drugs (nitroglycerin and aspirin) for acute myocardial infarction on rats were explored in this study, which provide reference for clinical practice.

The results of this study showed that the concentrations of HIF-1 and VEGF in the serum of rats with acute myocardial infarction were significantly higher than that of normal rats, suggesting that HIF-1 and VEGF may be involved in the occurrence of myocardial infarction. This was consistent with the study by Du *et al* on the concentration of HIF-1 α in rats with early acute myocardial ischemia (20). Further comparison of HIF-1 and VEGF levels between the routine therapy group and the study group showed that the content in the study group was higher than that in the routine therapy group, suggesting that the combination of atorvastatin and routine therapy increased the concentrations of HIF-1 and VEGF. HIF-1 is the key factor of cell regulation in hypoxia (21). HIF-1 can stimulate the release of VEGF-A when cells are anoxic. When cells are hypoxic, they produce HIF-1, which stimulates the release of VEGF-A. VEGF is the main downstream target gene of HIF-1α, which can promote neovascularization and make cells adapt to hypoxic environment. Therefore, we speculate that the activation of HIF-1α/VEGF signaling pathway after myocardial infarction stimulates angiogenesis, which plays a key role in the proliferation of myocardial tissue in patients with myocardial infarction. In the study on the effects of triple mutation HIF-1α on angiogenesis and cardiac function in rats with myocardial infarction, Li *et al* (22) found that triple mutation HIF-1α could improve angiogenesis and cardiac function, which was consistent with our conjecture. At the same time, we believe that the protective effect of atorvastatin on patients with acute coronary syndrome may enhance the stability of coronary atherosclerotic plaques (23) by reducing inflammatory cascade (24) and antithrombus activity (25), and may alleviate the possibility of thrombus embolism in the distal arteriole and alleviate myocardial remodeling and left ventricular function damage to a certain extent. We further studied the cardiac function of rats in each group. The results showed that there was no difference in the ventricular function between the study group and the model group before treatment, but the ventricular function in the study group was significantly higher than that in the model group and routine therapy group after treatment, suggesting that the combined treatment has better effect. Atorvastatin is a statin lipid-regulating drug (26), mainly acting on the liver, which can reduce the synthesis of cholesterol and has the effect of lowering blood lipid (27,28). Statins lipid-lowering drugs can improve the cardiac function of patients with acute myocardial infarction (29), and can be used for the treatment of the disorder of lipid metabolism in patients caused by acute myocardial infarction, which is beneficial to the treatment of acute myocardial infarction. Ielasi *et al* (30) found that atorvastatin combined with aspirin has a synergistic effect in the treatment of ischemic stroke, which can be a testament to our experimental results. The levels of HIF-1 and VEGF in rats of each group were further analyzed, and the levels of HIF-1 and VEGF in serum of rats with acute myocardial infarction were positively correlated with the time of treatment, negatively correlated with LVIDs and LVIDd, and positively correlated with LVEF and LVFS, suggesting that the recovery of myocardial function can be judged by measuring the concentration of HIF-1 and VEGF in peripheral blood in the future.

In this investigation, the concentrations and changes of HIF-1 and VEGF in rats with acute myocardial infarction were compared and analyzed after treatment of routine therapy alone and atorvastatin combined with routine therapy, and the therapeutic effect of atorvastatin combined with routine therapy on acute myocardial infarction was explored. However, due to the limited experimental conditions, there are certain limitations in this study. It was not proven that HIF-1 and VEGF are involved in the occurrence and progression of myocardial infarction. This can be a key direction of future research.

In conclusion, the concentration of HIF-1 and VEGF in serum of rats with acute myocardial infarction was significantly increased, and the concentration of HIF-1 and VEGF in the study group was significantly lower than that in the routine therapy group and the single drug group. Compared with routine therapy alone and the single drug, atorvastatin combined with routine therapy has more advantages in the treatment of myocardial infarction.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JY and HZ conceived and designed the study, and drafted the manuscript. JY, LZ and HZ collected, analyzed and interpreted

the experimental data, and revised the manuscript critically for important intellectual content. JY wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Zibo Central Hospital (Zibo, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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