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Study on the expression of VEGF and HIF-1α in infarct area of rats with AMI

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Abstract. – OBJECTIVE: To investigate the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α) in infarct area of rats with acute myocardial infarction (AMI).

MATERIALS AND METHODS: A total of 36 healthy male Wistar rats weighing 180 g-220 g were included in our study and were randomly divided into two groups of 6 rats each: sham operation group and experiment group. In sham operation group, surgery was performed by opening chest without ligation of arteria coronaria while, in the experiment group, surgery was performed to produce AMI model. Animals were sacrificed immediately after the operation on day 7 and day 14, respectively. The serum troponin, myocardial infarct area, microvessel density in infarct area, VEGF and HIF-1α expression were analyzed.

RESULTS: Differences in the serum troponin level, myocardial infarct area, microvessel density in infarct area, VEGF and HIF-1 expression level at different time points in sham and experiment groups had statistical significance ($p < 0.05$). On day 7, the serum troponin, myocardial infarct area, microvessel density in infarct area, VEGF and HIF-1 expression level were the highest and the level was second highest on day 14 while the levels were lowest immediately after the operation. The expression levels of VEGF and HIF-1α were positively related with the increasing density of microvessel in infarct area ($p < 0.05$).

CONCLUSIONS: The expression of VEGF and HIF-1α might be involved with myocardial remodeling and angiogenesis.

Key Words:
Acute myocardial infarction, Vascular endothelial growth factor, Hypoxia-inducible factor-1α, Microvessel density.

Introduction

Currently in the clinics, the major treatments for the Acute myocardial infarction (AMI) are focused on emergency vascular remodelling, such as coronary artery stent implantation or coronary artery bypass grafting. It has been suggested that in the pathogenesis of AMI the atheromatous plaque in lumen suffers from complete or incomplete acute block-up, which results in ischemia and hypoxia of the myocardium and thus the apoptosis or necrosis via relevant signal molecule pathway starts. Further molecular studies[1-3] showed that the blood supply of myocardium was mainly from a large amount of blood capillary, and the revascularization could only be applied to the blood vessel with diameter larger than 2 mm. Moreover, the pathophysiological process of blood capillary was significantly different from that of the large blood vessel. Under the stimulation of ischemia and hypoxia, blood capillary would secrete a large number of cytokines, such as vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α), inducing the regeneration of microvessels in infarct area, and reducing the degree of myocardial necrosis[4,5]. In our study, through constructing the AMI rat model, we have analyzed the relationship between the expression of VEGF and HIF-1α and the blood vessel regeneration under non-intervention at different time points.

Materials and Methods

Experimental Materials

All of the 36 healthy male Wistar rats, weighing 180 g-220 g, were provided by the Experimental Animal Center of our hospital and were divided into two groups: sham operation group and experiment group (6 rats in each group). Rats in sham operation group were operated by opening chest without ligation of arteria coronaria while rats in the experiment group, after building AMI model, were sacrificed immediately af-

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ter the operation on day 7 and day 14 respectively. The serum troponin, myocardial infarct area, microvessel density in infarct area, VEGF and HIF-1 expression level were analyzed.

Major reagents: CD34-antibody, HIF-1α monoclonal antibody, and VEGF monoclonal antibody were purchased from ZSGB Biotechnology Ltd. Co. Beijing, China.

AMI Model

As referred in the method of Drexler, the midpoint of left auricle and pulmonary cones were connected, and the left arteria coronaria were ligatured at 3 mm from the root of the coronary artery. Widening of the apex of heart and upward lifting of ST segment arch for over half an hour indicated that the infarction model was replicated successfully.

Detection Method

Orbital vein blood (0.5 ml) was collected from the rats. Troponin assay kit was used to detect via latex-enhanced immunoturbidimetric assay, and VEGF kit was used to measure VEGF content. All of the procedures were performed strictly according to the instructions provided.

Details of measurement on the area of myocardial infarction are as follows: media mix imaging analysis system was applied to analyze the images of the stained myocardial sections, measured the percentage of myocardial infarction area of each layer to the total left ventricular myocardium area of this layer, the percentage of total myocardial infarction area (AI) to the total area of the left ventricular myocardium (A) (Ai/A) x100% was calculated. CD34 primary antibody showed that if the nucleus and cytoplasm of the blood vessel are stained with yellow-brown, it indicates positive. A number of blood capillary on each section from three visual fields was calculated and the average value was taken to determine the density of blood capillary.

Western blot was used to detect the expression of HIF-1α. Details are as follows: DC Protein assay kit from Bio-Rad Company (Hercules, CA, USA) was used, and the absorbance values were measured by ultraviolet spectrophotometer at 570 nm, and the actual protein content was calculated according to the standard curve.

Statistical Analysis

Statistical software package SPSS 19.0 (SPSS Inc., Chicago, IL, USA) was applied to analyze the data; measurement data are presented as means±standard deviation; *t*-test was applied for comparisons between groups; variance analysis was applied for comparisons at different time points within the group; Pearson test was applied for correlation analysis; $p<0.05$ was considered with statistical significance.

Results

Comparisons of Troponin Levels and Myocardial Infarct Area

Troponin levels and myocardial infarct areas in experiment group were higher than those in sham operation group at different time points, and the difference had statistical significance ($p<0.05$). Differences in troponin levels and myocardial infarct areas at different time points in sham operation group had no statistical significance ($p>0.05$); whereas differences in troponin levels and myocardial infarct areas at different time points in experiment group had statistical significance ($p<0.05$). Troponin levels and myocardial infarct areas on day 7 were the highest, on day 14 were the secondly highest and right after operation were the lowest (Table I).

Table I. Comparison of troponin levels and infarct area.

	Troponin (μg/L)					Infarct area (%)				
Group	Post operation	7d	14d	F	*p*	Post operation	7d	14d	F	*p*
Sham operation group	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.347	0.238	0.06 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.326	0.455
Experiment group	0.16 ± 0.05	0.24 ± 0.06	0.18 ± 0.04	4.251	0.039	12.47 ± 3.15	16.29 ± 3.28	13.24 ± 3.63	4.628	0.037
t-value	6.217	6.954	6.138			6.598	6.634	6.745		
p-value	<0.001	<0.001	<0.001			<0.001	<0.001	<0.001		

Table II. Comparison of microvessel density in infarct area, VEGF and HIF-1α expression level at different time points in experiment group.

Time point	Post-operation	7d	14d	F	p
Microvessel density (number/vision field)	10.57 ± 2.32	16.23 ± 2.17	12.41 ± 2.06	5.746	0.024
VEGF	0.18 ± 0.05	0.63 ± 0.04	0.22 ± 0.03	5.639	0.026
HIF-1α	1.12 ± 0.07	1.74 ± 0.06	1.23 ± 0.05	6.105	0.019

Comparison of Microvessel Density in Infarct Area, VEGF and HIF-1α Expression Level at Different Time Points in Experiment Group

Differences in the microvessel density in infarct area, VEGF and HIF-1 expression level at different time points in experiment group had statistical significance ($p<0.05$). On day 7, microvessel density in infarct area, VEGF and HIF-1 α expression level were the highest, on day 14 being the second highest and after operation being the lowest (Table II).

Correlation Analysis of the Expression Level of VEGF and HIF-1α and the Increasing Microvessel Density in Infarct Area

Pearson correlation analysis showed that the expression level of VEGF and HIF-1α and the increasing microvessel density in infarct area were positively correlated (r=0.357, $p=0.042$; r=0.426, $p=0.037$).

Discussion

Hypoxia-inducible factor-1α (HIF-1α) is the nucleoprotein which is produced when myocardial cell is in hypoxia condition and is involved in the transcriptional regulation of many hypoxia responsive genes. The targeted genes of HIF 1α include vascular endothelial growth factor (VEGF) coding gene, erythropoietin (EPO) coding gene, heme oxygenase (HO-1) and Inducible NO synthases (iNOs) coding gene, glucose transporter protein1 (GLUT-1) and glycolytic enzyme-coding genes, including lactate dehydrogenase, phosphofructokinase, 3-glycerol phosphate dehydrogenase and so on[6]. VEGF is the most representative one, which could promote the blood vessel regeneration, so as to guarantee the microcirculation establishment during the recovery process of ischemic tissues[7]. Ischemia and hypoxia are strong stimulations of HIF-1α and VEGF, and Ca^{2+} overload, oxygen free radicals and various cellular factors are closely related to the generation of VEGF and HIF-1α. HIF-1α is the trigger factor of myocardial ischemia and reperfusion injury in a series of molecular reaction, which plays the core role in adaptive responses to ischemia and is considered to be the endogenous protective mechanisms initiating factor and common approach[8]. Regulation of the expression of HIF benefits the effect of on myocardial ischemic preconditioning and injury protection[9]. The study indicated that VEGF can reduce the apoptosis of myocardial ischemia-reperfusion injury by inhibiting the calcium sensitive receptor and play a role in promoting angiogenesis and myocardial protection[10].

In normal ventricular tissues, there is no HIF-la and VEGF mRNA expression. But in the specimen under acute ischemia or myocardial infarction advanced phase, HIF-1α and VEGF mRNA expression could be detected and the expression of HIF-1α appears earlier than the expression of VEGF[11,12]. Therefore, it was deemed that HIF-1α was one of the initiation factors of a series of molecular reactions after ischemia, which could also be regarded as the sign of time for AMI[13]. The expression of HIF-1α and VEGF after myocardial ischemia could promote the formation of ischemic myocardium and the new vessels to develop compensatory adaptation[14]. Local injection of naked DNA of the coded HIF-1α/VP16 into rabbit hind limb ischemia model could lead to the expression of VEGF to increase and promote the angiogenesis[15]. In another group, we made ligation of coronary artery on transgenic rats, which had HIF-1α gene that was regulated by α-myosin heavy chain promoter and found that transgenic rats could over express stable HIF-1α and significantly reduce the area of myocardial infarction after 4 weeks, and improve the cardiac functions. Moreover, the blood vessel density, VEGF, and iNOS expression in myocardial infarction area and the surrounding area of infarction were significantly improved[16,17].

The increasing expression of VEGF in the acute period of myocardial infarction was favorable for the regeneration of blood vessels, but unfavorable for the formation of functional vessels by increasing the vascular permeability. An increasing blood vessel density was the result of the interactions of a series of factors. Besides, other factors, such as HIF-1α, fibroblast growth factor, insulin-like growth factor 1 also played quite an important role in the process of angiogenesis[18-20]. The results of our study show the differences in the serum troponin level, the area of myocardial infarct, microvessel density in infarct area, VEGF and HIF-1 expression level at different time points in experiment group had statistical significance. On day 7, the serum troponin, myocardial infarct area, microvessel density in infarct area, VEGF and HIF-1α expression level were the highest, on day 14 were the secondly highest and after operation were the lowest. The expression levels of VEGF and HIF-1α were positively related with the increasing density of microvessel in infarct area. In our study, we compared the serum troponin level, area of myocardial infarct, microvessel density in infarct area, VEGF and HIF-1 expression level at different time points under non-interference and found that the regeneration ability of infarcted blood vessel on day 7 was the strongest, which might be because the expression level of VEGF and HIF-1α on day 7 were the highest. Of course, if we could make further analysis according to more segmented time points, we might get the optimal time window for more accurate blood vessel regeneration, thus providing a more accurate opportunity for clinical treatment.

Evidently, the results of our study have not been verified by HIF-1α gene transfection, nor involved in any specific downstream mechanism of HIF-1α and VEGF in promoting blood vessel regeneration. Thus, these problems still require further verification.

Conclusions

Our findings show that the expression of VEGF and HIF-1α in infarct area might be involved with myocardial remodeling and angiogenesis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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