·临床研究·

Expressions of HO-1 and VEGF in Patients of Sudden Cardiac Death and Their Significances

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ABSTRACT Objective: To investigate expression of the heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF) in the left ventricle in patients with sudden cardiac death (SCD). Methods: Immunohistochemistry and Simple PCI image analysis system were used to detect the expressions of HO-1 and VEGF in 33 cases of SCD and 18 cases of non SCD (control group). Results: The expressions of HO-1(155.090± 8.957) and VEGF(121.020± 10.927) in the myocardium of patients in SCD group were significantly higher than those in control group (116.200± 6.355, 84.207± 4.402 ,all $p<0.05$) . Conclusions: The high expression of HO-1 and VEGF may be related with the sudden cardiac death to some extent.

Key words: Sudden cardiac death; HO-1; VEGF; Immunohistochemistry

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Introduction

Sudden Cardiac Death (SCD) is caused by disease of cardiovascular system in a spurt, usually within six hours and some people suggest that it should be within twenty-four hours [1]. In 2006 the American Heart Institution, American Heart Association and Europe Heart Association defined SCD as natural death with sudden loss of consciousness which occured within one hour following acute symptoms[2]. Some research showed that the incidence of SCD was 41.84/100,000 and there were 544,000 people died of SCD in China every year according to the epidemiological study[3]. Coronary heart disease (CHD) was at the top of the SCD cause, secondly the myocarditis and hypertrophic cardiomyopathy [4]. Most people thought that the main cause of SCD was lethal arrhythmia induced by coronary artery stenosis or occlusion that led to myocardial ischemic-anoxic and local electrophysiology disorders. Now Routine pathological section observation is not reliable to diagnose SCD which increases difficulty to diagnosis and authenticate. The local ischemic-anoxic tissue can derive the overexpression of Heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF). This study used immunohistochemistry to detect the expression of HO-1 and VEGF in the SCD cardiac muscle, which may be the sensitive and specific index for diagnosing SCD.

1 Materials and Methods

1.1 Materials

Fifty-one heart specimens of the autopsy cases, from Department of pathology of Qingdao Municipal Hospital during 2006-2010, were studied in this study. Thirty-three patients (23 males，age range from 21 to 68 and 10 females, age range from 28 to 47) were included in the SCD group, of which twenty-eight cases died of coronary heart disease and five cases died of cardiomyopathy. All the cases were diagnosed as SCD by observing the HE staining slides. Eighteen patients (12 males ,6 females age range from 29 to 55) were included in the control group. All the patients were caused by traffic accident or high fall injury without heart disease. Rabbit anti-human HO-1 Polyclonal Antibody (Bejing Biosynthesis Biotechnology CO. LTD), working concentration: 1: 400. Mouse Anti-human VEGF Monoclonal Antibodies (ready-to-use, Maixin biotechnology CO.LTD); Ready-to-use immunohistochemistry kit (Maixin biotechnology CO.LTD) .

1.2 Methods

1.2.1 Sample disposal One piece of tissue (size 1.5× 1.5× 1.5 mm^3) were took from the apical area, and the samples were fixed in 10% neutral formalin for 12h. Pathologic Serial Section was applied to each specimen. One slide was used for HE staining and the other two were used for HO-1 and VEGF immunohistochemical staining.

1.2.2 Immunohistochemical and routing staining method The PV-6000 immunohistochemical method was used according to the kit introduction. The positive slide supplied by the kit was used as positive control and PBS as negative control.

1.3 Analysis of Results

1.3.1 Analysis of Immunostained Sections Positive staining was defined as the brown granules in the cytoplasm or/and nucleus.

1.3.2 Image analysis HO-1and VEGF was evaluated quantita-

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tively according to SIMPLE PCI image analysis system. For each section, five high-power microscope visual fields were randomly selected and brown granules presence in the cytoplasm defined as positive staining. The IOD detected by the image analysis system reflexes the protein level[5].

1.3.3 Statistical analysis SPSS16.0 statistical package and t-Test were used. P-values of <0.05 were considered as statistically significant.

2 Results

2.1 HE staining results

SCD group: There were some pathological features of these cases such as coronary artery stenosis or occlusion, some cases with cholesterol crystals and some with thrombus in the lumen. Myocardial hypertrophy, myocardial fibers breakage, corrugated deformation, increase of fiber connective tissue, interstitial hemorrhage and edema, and inflammatory cells infiltration were present in these cases. Pathological examination also found infiltration of fatty tissue into the cardiac muscle.

Control group: Microscopy revealed no coronary artery stenosis or occlusion in the control group of which some slides showed breakage of myocardial fibers.

2.2 Results of Immunostaining Sections

2.2.1 Expression of HO-1 and VEGF in cardiac muscle The expression of HO-1 in the cytoplasm in the SCD group was higher (Fig.1a) and sparse staining showed tiny brown granules in the nucleus. The expression of HO-1 in the cytoplasm or/and nucleus in control group was evaluated as positive/negative (Fig.1b). The expression in SCD group was significantly stronger than that in the control group ($p<0.05$).

The expression of VEGF: In the SCD group twenty-nine cases showed coarse brown granules which was defined as strong positive, and there were expression in the cytoplasm in the SCD group of which four cases showed negative (Fig.2a). There were three cases in the control group showed weak expression in the myocardial and vascular endothelial cell membrane (Fig.2b). The expression of VEGF in the SCD group was significantly higher than that in the control group ($p<0.05$).

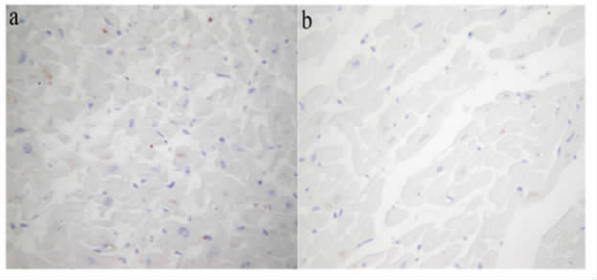

Fig 1 Expression of HO-1 in the left ventricle SCD group (a) and control group (b)(200×)

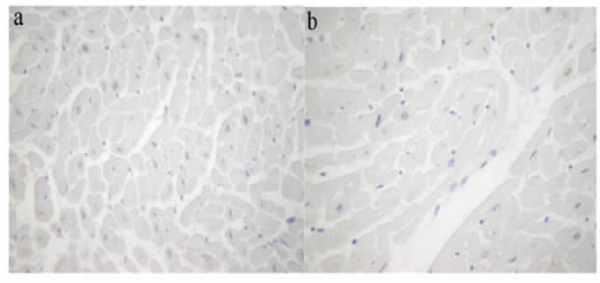

Fig 2 Expression of VEGF in the left ventricle SCD group (a) and in the control group (b)(200×)

2.2.2 image and Statistical analysis The expression of HO-1and VEGF in the SCD group was significantly higher than that in the control group ($p<0.05$) (Table 1).

Table 1 IOD of HO-1 and VEGF (IOD, $\bar{x}\pm s$)

groups	N	HO-1 (IOD)	VEGF(IOD)	t	p
control	18	116.200± 6.355	84.207± 4.402	16.989	0.023
SCD	33	155.090± 8.957	121.020± 10.927	17.031	0.020

3 Discussion

3.1 SCD

The pathogenesis of SCD is complicated, most viewpoints thought myocardial ischemia and hypoxia caused by abrupt cessation of myocardial blood flow as the major cause of the sudden cardiac death. Myocardial acute ischemia resulted in the obvious changes of metabolism between the ischemia and perfusion legions that cause redistribution of a number of ions, including H^+, Na^+, Ca^{2+}, and K^+across the myocardial cell membrane, an event that has profound electrophysiological consequences through its influence on the activity of a variety of ion channels and transporters. Net cellular K^+ loss and subsequent extracellular K^+ accumulation during acute myocardial hypoxia and/or ischemia causes sustained membrane depolarization that leads to a slowing of conduction and altered refractoriness, which in combination with other factors promote VT/VF and SCD[6].

3.2 HO-1 and SCD

There are three forms (HO-1、HO-2、HO-3) of the HO in the body which is the rate-limiting enzyme in heme degradation. High level of inducible HO-1 induced by hypoxia, hyperxia, heavy metal, endotoxin and other acute and chronic oxidative stress states

can be as the oxidative stress marker. Oxidative stress is the pathophysiological basis of many serous diseases, HO-1 can play a protective role in cells damage mediated by oxidative stress. Brouard S found that HO-1 in culture endotheliocytes could protect cells from apoptosis. This effect was dependent on the ability of CO generated by HO-1 to activate the p38 MAPK signal transduction pathway[7]. This study showed that the expressions of HO-1 protein (155.090± 8.957)in the myocardium of patients in SCD group were significantly higher than those in control group (116.200± 6.355 ,p<0.05) . The overexpression of HO-1caused by HO-1 inducer or transgenic technology can significantly enhance the antioxidant damage function, inhibit inflammatory reaction, reduce ischemia- reperfusion injury and has obvious protective effect on ischemia- reperfusion tissue[8], but the mechanism is not very clear. The results demonstrate that the difference of the quantity and form of HO-1 expression may have relation with SCD provoked by myocardial ischemia.

3.3 VEGF and SCD

Many studies have focused on the expression of angiogenic factors after infarction, especially VEGF and its receptors. VEGF is the specific mitogen of the endothelia cells and it has strong effect of promoting vascular endothelial cell proliferation. Recent evidences suggest that VEGF may, in addition to promote angiogenesis, modulate various aspects of endothelial function and repair, leading to cardioprotection [9-13]. It has been reported that it generally distributed in the body and the gene and the protein of VEGF has been detected in the heart, brain, kidney, lung, liver. Under normal circumstances, however, the contribution of neoangiogenesis to the infarct-capillary bed network is insufficient to keep pace with the tissue growth required for contractile compensation and is unable to support the greater demand of the hypertrophied but viable myocardium.This study found that the expression of VEGF was significantly higher in SCD group than that in the control group by image quantitative analysis and data processing. The overexpression of VEGF was considered to be the result of the VEGR receptor increase on injury cadiocytes and vascular endothelial cells induced by local myocardial ischemia and hypoxia[14]. Some experiments discovered that if the myocardial ischemia lasted for 5-10min there would be the expression of VEGFmRNA in the myocardial cell which reached peak in 30 min, and the level of VEGFmRNA in myocardial cell could increase 3-5 times [15]. VEGF upregulation has been previously demonstrated in animal models subjected to environmental hypoxia [16]. The increasing of VEGF tissue levels became detectable 6 hours after application of the hypoxic challenge, and the present study demonstrated that the VEGF response to a short hypoxic exposure would peak at 12 hours and return to baseline within 24 hours. It has been documented that a period of one to two weeks of VEGF overexpression, mediated by direct intramyocardial gene transfer, may be sufficient to induce collateral vessels in ischemic myocardium[17].

3.4 HO−1 and VEGF

It is known that VEGF administration restores normal vascular responsiveness in ischemically injured tissue[18]. Recently, studies have implicated a role for HO-1 in angiogenesis. The study of Lin HH showed that HO-1 gene transfer post myocardial ischemia (MI) provided protection at least in part by promoting angiogenesis through inducing angiogenic growth factors [19]. Some literature reported that HO-1 could promote the expression of VEGF and enhance the sensitivity of the receptor [20]. The CO generated by HO-1 can markedly stimulate the synthesis of VEGF and its receptor in the environment of 0.1 %[21], so it can be speculated that the level of HO-1 regulates the expression of the VEGF and its receptor in the situation of ischemia and hypoxia. Soares et.al[22] demonstrated that HO- 1 had the effect of anti apoptosis but the mechanism of the SCD genesis and development was not clear . Further studies are needed. The combination of HO-1 and VEGF provide a new tool for the SCD diagnosis.

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心脏性猝死者心肌 HO-1 和 VEGF 的表达及意义

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摘要 目的:探讨血红素加氧酶 -1 (heme oxygenase-1,HO-1)、血管内皮生长因子(vascular endothelial growth factor ,VEGF)在心脏性猝死病人心室肌细胞中的表达及其意义。**方法**:运用免疫组织化学方法和 Simple PCI 图像分析系统观察 33 例心脏性猝死组和 18 例非心脏性猝死对照组尸检心肌组织中 HO-1、VEGF 蛋白的表达情况。**结果**:心脏性猝死组心肌组织 HO-1(155.090± 8.957) 和 VEGF 蛋白表达(121.020± 10.927)均显著高于非心脏性猝死对照组(116.200± 6.355 、84.207± 4.402 ,均 $p<0.05$)。**结论**:HO-1 和 VEGF 蛋白在心脏性猝死者心肌组织表达增强,可能与心脏性猝死有一定关系。

关键词:心性猝死;血红素加氧酶 -1;血管内皮生长因子;免疫组织化学

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