

Original Article

Comparison between myocardial infarction and diabetes mellitus damage caused angiogenesis or energy metabolism

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Abstract: This study aims to compare and analyze lactate dehydrogenase (LDH), succinic dehydrogenase (SDH) and differences in capillary density level in the model of myocardial damage which caused by rats diabetes. The Wistar rats were divided into 4 groups, including control, diabetic, myocardial infarction and two diseases combined group. Ligate descending branch of left coronary artery on 1/3 position or inject streptozotocin into abdominal cavity to establish two kinds of disease models. After 6 w, obtain the myocardial tissues to do the vascular density analysis of tissue sections which are stained and cell tissue enzyme. Explore change of relevant index and differences among groups. Results indicated that degree of LDH and SDH decrease in two kinds of disease model. Compared with control group, level of myocardial vascular of myocardial injury group is higher, and diabetic group is higher than non diabetic group. Quantitative result of FFA in mitochondrial suspension of single disease group is higher than that of control group and two diseases combined group. Level of FFA and LDH of two diseases combined group is consistent with control group. In conclusion, after myocardial damage, which is caused by diabetes mellitus or myocardial infarction, degree of local vascularization increases, diabetes mellitus is more obvious. After myocardial damage, process of myocardial mitochondrial glycolysis and oxidative phosphorylation has some obstacles. But these two kinds of diseases all have cardiac muscle cell which can keep generated procedure of aerobic and anaerobic energy to instead the normal function of cardiac muscle.

Keywords: Myocardial infarction, diabetes mellitus, myocardial damage, energy metabolism, revascularization, comparative analysis

Introduction

Myocardial hypertrophy is one of the typical myocardial morphological changes when chronic myocardial function changes. The level of cell energy metabolism disorder shows that hypertrophic cardiomyopathy myocardial dysfunction has developed to a more serious stage [1]. The compound and usage of ATP needs to be based on the structure and function of normal cells which is consistent with morphological data of ischemic cardiomyopathy and diabetic cardiomyopathy with myocardial metabolic disorder ability. And finally lead to the deterioration of the disease and the irreversible development [2-4]. Normally, the energy metabolisms of myocardial cells are mainly provided by del-spray and partial oxidation of glucose. In the aspect of oxygen consumption, ischemic heart

failure tends to oxidation of glucose. Del-spray is the main alternative substance for diabetic patients, mainly because there are some barriers in the transportation of insulin -sensitive glucose. A lot of acetylcoenzyme A enter and gather in mitochondria which will restrain the complex function of pyruvate dehydrogenase [5, 6]. At present, it is still controversial between the energy generating type foundation and clinical data. The upregulation of apoptosis activity is the main adaptability of cardiac muscle. On the other hand, the microvascular generation mainly makes up for the vacancy between oxygen demand and oxygen supply in cardiac muscle cell [7]. The main object of this study is that to explore the differences of angiogenesis and Chronic changes in energy metabolism after myocardial damage which is caused by myocardial infarction and diabetes mellitus.

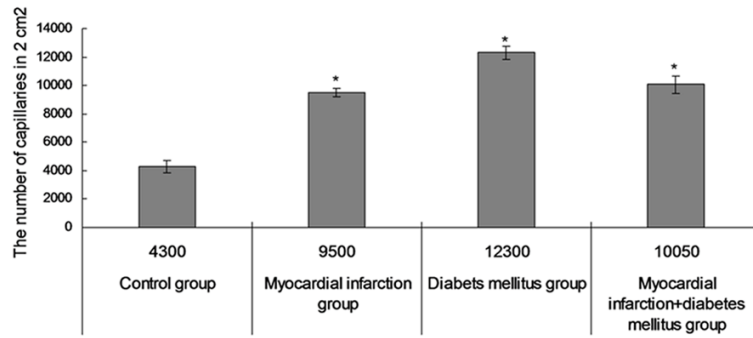


Figure 1. The capillary density distribution of each group. * $P < 0.01$ compared with control group.

Materials and methods

Construction of animals and models

Divide 20 adult Wistar rats (average weight is 250-300 g) into 4 groups: blank control group (group 1), myocardial infarction group (group 2), diabetes mellitus group (group 3) and myocardial infarction combined with diabetes mellitus group (group 4). To establish the model of myocardial infarction, anaesthetize the rats with aether and ligate 1/3 on the left anterior descending coronary artery [6]. After myocardial infarction, judge the change of cardiac muscle by morphologic characteristics and make the follow-up examination in 6 w after coronary occlusion.

Diabetes is induced with a single intraperitoneal injection of streptozotocin (Sigma, USA), the dosage is 60 mg/kg which is dissolved in 0.01 mol/L citrate buffer solution (pH=4.5). The index of building diabetes models are 3-4.5 times of blood sugar level, polydipsia, diuresis and weight reduction. Inject streptozotocin for 6 w will be included in the study. For the two diseases combined group, the rats will be injected streptozotocin into their abdominal cavity after blocking coronary artery for 4 w and it will be included in the study after being observed for 2 w (the development period of total diseases are all 6 w). All the rats are raised in animal experimental center. All the operations are admitted by ethics committee of our hospital and all the animals are euthanized by anaesthesia.

Vascular density examination

Get myocardial tissue from the animal left ventricular scar of group 1 and group 2. Group 1

and group 3 will their corresponding anatomical organization. Put and fix all the tissues in 10% neutral formalin and then do the embedding treatment for tissues according to the standard method. In order to evaluate myocardial revascularization, use methenamine silver (P.A.S.M. reagent kit) to stain tissue slice (thickness of slice is 4-5 μ m). For each slice, random select 10 to enlarge 100 times and count the number by using AxioLab A1 microscope (Carl Zeiss, Germany). Finally, regard the arithmetical mean as vascular density value.

Enzymology research

Use the slice which the thickness is 10 μ m to cut the frozen left ventricular tissue into slices in continuous cryostat TP-OM-5-01 (Russia). Expound the mechanism of succinic dehydrogenase (SDH) and lactate dehydrogenase (LDH) by histochemical reaction [7]. Transfer light source which the wavelength is 546 nm by light microscope "Lyumam-IZ" (Russia) to quantify the amount of enzyme reaction. Measure the transmittance density of at least 50 different cardiac muscle cells. Get the homogenate in cardiac muscle cell and mitochondria in sucrose containing medium by differential centrifugation. The culture medium contains 250 mM sucrose, 10 mM EDTA, and 10 mM HEPES, pH=7.4. The mitochondria suspends and is kept in the solution which contains 250 mM sucrose, 10 mM HEPES, pH=7.4 [8]. Use photoelectric colorimetric analyzer to evaluate the plasma content of free fatty acids (FFA) and myocardial homogenate and mitochondria which are contained in soliquoid. Use Diagnostic kit system DiaSys (Germany) to quantify the content of FFA.

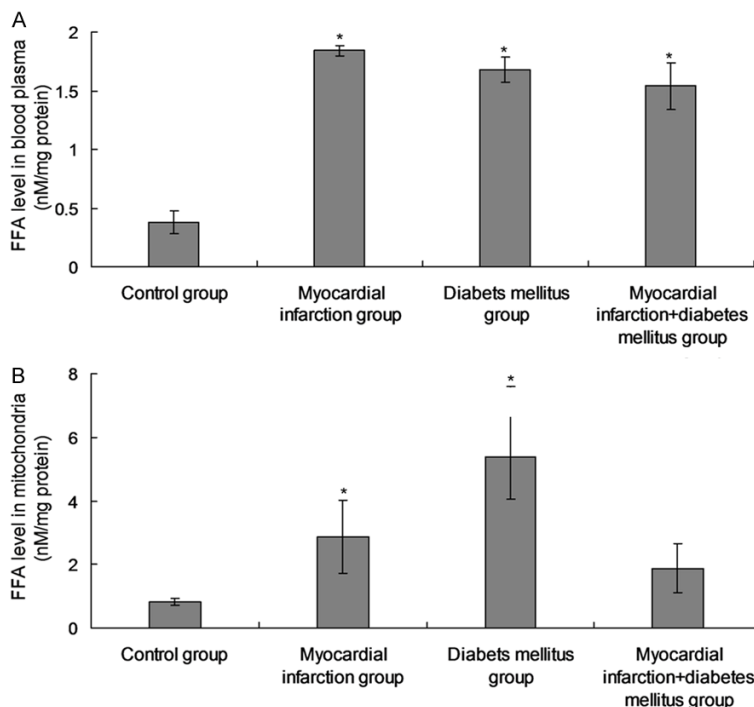
Statistical analysis

Use SPSS 16.0 for data statistics and analysis. The data that are acquired in this study are in abnormal distribution (Shapiro-Wilk test, $P > 0.05$). Considering the quantity of samples in the group is small and independent, so use Mann-Whitney non-parametric test to analyze. Use Wilcoxon to test in groups because of the

Table 1. The FFA levels in blood plasma, myocardial homogenate and myocardial mitochondria ($\bar{X} \pm m$, nM/mg protein)

Animal group	Blood plasma	Homogenate	Mitochondria
1. Control group	0.38 ± 0.1	1.02 ± 0.14	0.83 ± 0.12
2. Myocardial infarction group	$1.84 \pm 0.04^*$	1.19 ± 0.14	$2.86 \pm 1.15^{* \#}$
3. Diabetes mellitus group	$1.68 \pm 0.11^*$	1.51 ± 0.17	$5.83 \pm 1.31^{* \wedge}$
4. Myocardial infarction+diabetes mellitus group	$1.54 \pm 0.20^*$	1.35 ± 0.15	$1.88 \pm 0.78^{* \# \wedge}$

*P < 0.01 compared with control group, #P < 0.01 compared with diabetes mellitus group, ^P < 0.01 compared with myocardial infarction group.

**Figure 2.** The FFA level in blood plasma and mitochondria. A. The FFA level in blood plasma. B. The FFA level in mitochondria. *P < 0.05 represents the FFA level compared to the control group.

Quantitative detection of free fatty acids (FFA)

Table 1 shows the content of FFA in samples. For the groups which have pathological changes, the FFA content in plasma increases. Differences among groups have no statistical significance. The differences of myocardial homogenate among groups also have no statistical significance. Quantitative result of FFA in the mitochondrial suspension shows that all the groups are significantly different. The FFA content in blood plasma of group 2 (myocardial infarction group) and group 3 (diabetes mellitus group) are higher significantly compared to the control group (**Figure 2A**, $P < 0.05$). Meanwhile, the FFA level of group 4 (diabetes mellitus and myocardial infarction

group) is higher significantly compared to the control group (**Figure 2A**, $P < 0.05$).

Furthermore, The FFA content in mitochondria of group 2 (myocardial infarction group) and group 3 (diabetes mellitus group) are higher significantly compared to the control group (**Figure 2B**, $P < 0.05$). However, the FFA level of group 4 (diabetes mellitus and myocardial infarction group) is consistent with the control group (**Figure 2B**, $P > 0.05$).

Comparison among groups for LDH and SDH of tissue enzymes

The research result of histoenzymology shows that LDH activity level of cardiac muscle cell of

variable quantity. Analyze and evaluate morphological data by single argument. If $P < 0.05$, the difference has statistical significance.

Result

Quantify blood vessel density

The data shows that the capillary density of the control group is the lowest (**Figure 1**), the capillary density of group 3 is the highest which has significantly difference compared with other groups. The capillary density of the two diseases combined group is obviously higher than that of control group and myocardial infarction group.

Table 2. Comparison of myocardial tissue activity for each group ($X \pm m$)

Animal grouping	LDH	SDH
1. Control group	0.73 ± 0.04	0.51 ± 0.02
2. Myocardial infarction group	$0.41 \pm 0.01^*$	$0.32 \pm 0.01^{*,\#}$
3. Diabetes mellitus group	$0.37 \pm 0.03^*$	$0.20 \pm 0.02^{*,\wedge}$
4. Myocardial infarction+diabetes mellitus group	$0.62 \pm 0.03^{\#, \wedge}$	$0.78 \pm 0.05^{*, \#, \wedge}$

*P < 0.01 compared with control group, #P < 0.01 compared with diabetes mellitus group, ^P < 0.01 compared with myocardial infarction group.

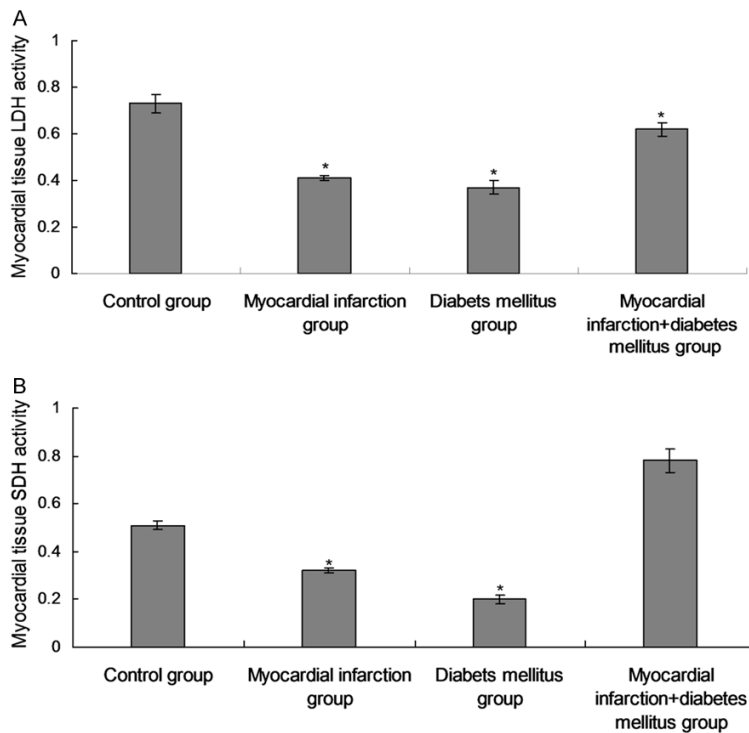


Figure 3. The LDH and SDH activity in myocardial tissue. A. LDH activity. B. SDH activity. *P < 0.05 represents the LDH or SDH activity compared to the control group.

the rats that have one single disease is more obviously higher than that of control group (Table 2), which means energy production decreases in the anaerobic environment. While the evaluation result for LDH of group 4 is consistent with that of control group, which means the normal glycolytic cycle is still kept in the two diseases combined group. The results indicated that the LDH activity in group 2 and group 3 are lower significantly compared to the control group (Figure 3A, $P < 0.05$). Also, the LDH level in group 4 is lower significantly compared to control group (Figure 3A, $P < 0.05$).

Furthermore, the SDH activity in group 2 and group 3 are also lower significantly compared

to the control group (Figure 3B, $P < 0.05$). The data shows that compared with control group, the SDH activity level of group 2 and group 3 is decreased 1.5 times and 2.6 times respectively (Figure 3B). However, in group 4, the SDH level is 2.5 times and 3.5 times compared to that of single disease model group and control group respectively (Figure 3B).

Discussion

At present, myocardial infarction and diabetes mellitus are the common clinical diseases which can cause myocardial damage and affect cardiac function. So the related researches are always the key of basic and clinical study [8]. After myocardial damage, the angiogenesis may exist in the local ischemic lesions to increase the local oxygen supply,

make up for the oxygen demand of myocardial cells and the vacancy of the oxygen supply to instead physiological function of cardiac muscle. After myocardial infarction, in the aspect of oxygen consumption ischemic heart failure tends to oxidation of glucose. Fatty acid is the main alternative substance for diabetics. So it is of great significance to study on the extent of myocardial blood vessels at the condition of myocardial damage which is caused by the two diseases and energy metabolism [9, 10].

In this study, the content of FFA in plasma for the group which has pathological change increases. That shows that with the pathologi-

cal change, cardiac muscle cell still can effectively control the absorbing of FFA for their protection mechanism. It can be speculated that this effect exists in the process of human chronic diseases. While in different disease models, the ability of mitochondrial fatty acid oxidation are different. The content of FFA is the highest in floating liquid which is 5 times than that of control group, the level of myocardial infarction group is about 3 times than control group, but the FFA level of the two diseases combined group is consistent with that of control group. The oxidation process of FFA needs to depend on oxygen supply. So in this case, it is important to produce energy in the anaerobic environment. The result of histoenzymology shows that the activity level of rats cardiac muscle cell which has one single disease is obviously higher than that of control group, which points that the reduction of energy production in the anaerobic environment. While the evaluation result of group 4 is consistent with that of control group, which shows that it can keep the normal glycolytic cycle in the two diseases combined group. At the same time, the data shows that compared with group 2 and group 3, SDH activity level of control group decreases 1.5 times and 2.6 times respectively. SDH level of group 4 is 2.5 times and 3.5 times than that of one single disease group and control group respectively. The result shows that in one single disease model, the oxygen demand and the production of anaerobic energy decrease after myocardial damage. But still keep aerobic energy production process for diabetes mellitus and myocardial infarction group, even the production of ATP increases. The data can be explained by energy metabolism adaptive remodeling. Especially the process of energy production in the myocardial mitochondria can be switched to oxidative dehydrogenation of succinate pathway. According to the existing reports, butanedioic acid can be activated to oxidation process in tissue hypoxia environment [11-14].

The enhanced aerobic process is related with the enhanced activity of new vessels to increase myocardial flow accordingly. In this study, the capillary density of control group is the lowest while group 3 is the highest. Compared with other groups, there are significant differences. The vascular density of the

two diseases combined group is obviously higher than that of control group and myocardial infarction group. So no matter diabetes mellitus, myocardial infarction or the combination of these two diseases, they all can promote the increasing of myocardial revascularization level. The specific endogenous mechanism is different in these two disease models. The reason is that vascularization level of myocardial infarction group is not the highest may due to local vascular bed damage. So we can find the formation of collateral circulation in injured parts. Multiple organ microvascular disease is the typical clinical manifestation of type I diabetes mellitus which also can be the main core mechanism of vascularization after myocardial damage [15-18]. The vascularization level of the two diseases combined group is lower than that of diabetes mellitus group, while it is higher than that of myocardial infarction group which can confirm this assumption indirectly. The data shows that the increase of myocardial capillaries density plays an important role in the anaerobic energy production of the two diseases combined group. But in the study, we find no direct correlation between vascular density and SDH. Besides the blood vessel density, the activity of LDH decreases in the two single-disease groups while increases in the two diseases combined group. So the increased myocardial blood flow is not the only expression of the oxygen demand and anaerobic energy production in myocardial mitochondria.

In conclusion, cardiac muscle cell can keep the oxygen demand and anaerobic energy production process at the condition of the combination of specific pathology to effectively use amylaceum and del spray to transport energy to the normal function of the myocardium [12]. The abnormal energy metabolism may be related with adaptive activation of SDH. It may be consistent with the function of adapting and keeping cellular energy production by SDH or succinic acid generation and oxidation process change.

Disclosure of conflict of interest

None.

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