Effect of Resistin on the Heart Structure of Mouse

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ABSTRACT

Background: In recent years, relevant studies have shown that a large number of adipokines were involved in energy homeostasis in the body, regulating the balance of glucose and lipid metabolism. Resistin was a special type of adipose factor and its discovery and related studies have shown a close relationship between resistin and heart disease. However, there was relatively little research on the structure of heart tissue, so we conducted this present study.

Methods: In order to study the effect of resistin on the heart structure of mice, 20 healthy C57BL/6 mice were randomly divided into experimental and control groups (n=10). The mice of the experimental group were injected with 20 μl/d of resistin solution through the tail vein. The mice of the control group were injected with the same volume of double distilled water. HandE staining, Wheat Germ Agglutinin (WGA) staining, Masson’s trichrome staining and electron microscopy were used to observe the changes of microscopic structure and ultrastructure of mouse cardiomyocytes.

Result: The results of HandE staining showed that the myocardial cells in the experimental group were disordered, the cytoplasm was loosely distributed and the gap between adjacent cells increased. WGA staining showed that the fluorescence green intensity of the mice in the experimental group was higher than that of the control group. The myocardial cell membrane thickened, the average cross-sectional area of cardiomyocytes was larger than that of the control group. The results of the Masson staining showed that the staining of the cytoplasm of myocardial cells in the experimental group was significantly shallow and the blue structure was significantly increased. The electron microscopy showed that the number of mitochondria decreased and the mitochondria lightly dissolved or disappeared. This study confirmed that resistin could change the content of collagen fibers and myofibrils in myocardial cells, induce cardiac hypertrophy and cause certain damage to mitochondria of cardiomyocytes. It was speculated that it could affect the physiological function of cardiomyocytes to some extent.

Key words: Heart, Mouse, Resistin, Structure.

INTRODUCTION

Resistin, known as adipocyte-specific secreted Factor (ADSF) was discovered in 2001 (Steppan et al., 2001). After that many researcher began to study it. Resistin belongs to the resistin-like family and is a cytokine secreted by cells of adipose tissue. It contains rich cysteine and serine residues, is a new peptide hormone expressed only in white adipose tissue (Bengrong and Huacing, 2003). Resistin has many effects on cardiovascular system, including inducing endothelial dysfunction, promoting myocardial ischemia reperfusion injury, etc. However, there are different views on ischemia reperfusion. The research has shown that resistin has protective effects on myocardial ischemia reperfusion injury (Gao et al., 2007) and resistin can aggravate myocardial ischemia reperfusion injury (Rothwell et al., 2006), further research will be needed to confirm this difference. Kim et al. (2008) reported that resistin overexpression altered cardiac contractility, conferred to primary cardiomyocytes of all the features of the hypertrophic phenotype. In a multi-ethnic cohort free of cardiovascular disease at baseline, elevated resistin levels were associated with incident heart failure (Cai et al., 2022). The serum resistin level in patients with chronic heart failure increased and the increased resistin level becomes more obvious with the deterioration of cardiac function (Wei et al., 2016). The study revealed that diminution in circulating resistin reduced myocardial fibrosis and apoptosis and improved heart functions in a mouse model of heart failure. The results of this study indicated that controlling resistin levels may provide a potential therapeutic approach for treating pressure overload-induced heart failure (Zhao et al., 2022). Resistin impaired glucose uptake in cardiomyocytes by mechanisms that involve altered vesicle trafficking (Gravelleau et al., 2005). Glucose uptake in cardiomyocytes was reduced by resistin upregulation (Wang et al., 2007). Many scholars had studied the relationship between resistin and heart disease, but there were few reports on the effect of resistin on heart tissue structure. The present study attempted to observe the variation in the structure of the mice heart after injection resistin. The results may offer morphological data and theoretical basis to study the effect of resistin on cardiac function in mice.
MATERIALS AND METHODS

Animals
Ten apparently healthy C57BL/6J mice were selected and placed in the experimental animal room, one for each cage and numbered. Adaptive feeding and watering was carried out for 4-5 days and the weight of the mice was recorded daily. The mice were then randomly divided into two groups, 10 mice as a experimental group and 10 mice as a control group. The mice in the experimental group were injected with 20 μL/d through a tail vein, while those in the control group were injected with the same volume of double distilled water.

Sample collection
Centrifuge tubes of appropriate size were prepared and marked before treatment and glutaraldehyde and paraformaldehyde fixing solution were added into different tubes respectively and stored at -4°C refrigerator. The mice were euthanized on the sixth day after injection (note: 12 h of food and water should be cut off before treatment), then the heart sample were collected. The heart was cut into two pieces and the long strip of myocardium was put in 4% paraformaldehyde-phosphate buffered solution and the other piece was put in 3% glutaraldehyde.

Wheat germ agglutinin (WGA) staining principle
Lectins are a class of proteins that can bind to sugars specifically and reversibly. Wheat Germ Agglutinin, one of these lectins, has a molecular weight of 43.2KD. This lectin can specifically and inversely bind to a glycoprotein on the myocardial cell membrane. The myocardial cell membrane is stained green and is compared with the control group to see if the myocardial cell is hypertrophy. Or in order to be more accurate, special software can be used to measure the diameter and area of the stained cells, so as to quantitatively analyze whether the cardiomyocyte hypertrophy (Sun, 2013).

Masson’s trichrome staining principle
The principle of this method is related to the size of anionic dye molecules and the permeability of tissues. The size of molecules is reflected by their molecular weight and small molecular weights are easy to penetrate tissues with dense structures and low permeability; however, high molecular weight can only enter structurally loose and highly permeable tissues. However, light green or aniline blue have large molecular weights, so after Masson’s staining, muscle fibers appear red, while collagen fibers appear green or blue, mainly used to distinguish between collagen fibers and muscle fibers.

Electron microscopy
Heart tissue blocks were fixed in 3% glutaraldehyde; processed by standard methods through secondary fixation in 1% osmium tetroxide, dehydrated through a graded series of ethanol and subsequently embedded in resin. Ultra-thin sections were cut and stained conventionally with uranyl acetate and lead citrate, viewed and photographed in transmission electron microscopy (Liu and Ju, 1996).

Statistical analyses
Results are reported as mean±standard error (S.E.). Significant differences between means were analyzed with one-way analysis of variance via SPSS 17.0 followed by Duncan’s post hoc test. Statistical significance of difference was set at p<0.05.

RESULTS AND DISCUSSION

Effect of resistin on mice heart tissue stained with H and E
In the control group (Fig 1-A, Fig 1-C, Fig 1-E), cardiomyocytes were arranged in a regular way and the size of cardiomyocytes and their interstitial tissue distribution was normal. The cardiomyocytes in the experimental group of mice (Fig 1-B, Fig 1-D, Fig 1-F) were disorganized, with...
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Note: Control group, mice were injected with double distilled water; Experimental group, mice were injected with resistin.

Fig 3: Effect of resistin on mean cross-sectional area of myocardium.

loosely distributed cytoplasmic staining, normal capillary structure, blank areas in the cytoplasm and enlarged gaps between adjacent cells. There are many factors that can cause changes in the structure and function of the heart, which will lead to the occurrence of heart disease. Research reports that cardiac dysfunction in obesity is caused by lipolysis and is prevented by reducing cardiac lipids (Zhou et al., 2000). Xin mentioned in her study that adipose tissue can provide energy for the body, of which more than 90% is triglyceride, but excessive fat will cause a certain burden on the body (Xin, 2014). The results of this study showed that after the injection of resistin in mice, the myocardial cells were arranged in disorder, the adjacent cell space was enlarged, the cytoplasm was loose and there was a blank area in the cytoplasm. After the HandE staining of adipocytes, the cytoplasm fat dissolved and vocalized, which was presumed that resistin could increase the ability of cardiac fat synthesis of mice.

Effect of resistin on mice heart tissue stained with WGA

The fluorescence green intensity of experimental group (Fig 2B, Fig 2D, Fig 2F) was significantly higher than that of control group (Fig 2A, Fig 2C, Fig 2E). The myocardium of experimental group was thickened and myocardial hypertrophy was observed. The mean cross-sectional area of myocardial cells in experimental group was higher than that in control group (Fig 3). Luo et al. (2016) mentioned that resistin can cause hypertrophy of H9c2 cardiomyocytes, indicating that after injection of resistin, the surface area of cardiomyocytes is significantly increased, which can change cell cycle and promote protein synthesis (Jianwei, 2015). In this study, the cell membrane was stained with fluorescent green by WGA staining, which showed that the cell membrane of mice after the injection of resistin was thickened and the average value of the cross-sectional area of myocardial cells was higher than that of normal mice. It was confirmed that after the injection of resistin in mice, the cardiac tissue structure changed and cardiac hypertrophy occurred. The study found that resistin can affect the occurrence and development of cardiac hypertrophy through different ways (Yan et al., 2022; Liu et al., 2016; Luo et al., 2016). Previous researches showed that resistin could directly regulate the endothelial cell function, make the endothelium induced and activated and increase the mRNA expression and promoter activity in the endothelial cells, resulting in the deposition of lipids under the intima of blood vessels, the formation of lipid stripes, the damage of the intima, the thickening of the vascular wall and the occurrence of secondary fibrosis (Calabro et al., 2004; Yanjie and Hui 2009). As far as cardiac hypertrophy is concerned, there are at least two mechanisms: one is the mechanical extension of the heart itself and the other is that the expression of some contractile protein genes is affected by the activation of surface receptors.
Effect of resistin on Masson staining of mouse heart tissue

In the control group (Fig 4A, Fig 4C, Fig 4E), the cytoplasm of myocardial cells was red with uniform distribution, with regular arrangement of muscle fibers and fewer structures were blue. While in experimental group (Fig 4B, Fig 4D, Fig 4F), the cytoplasmic staining of myocardial cells was significantly lightened and the blue structures were significantly increased. Masson staining showed that collagen fibers were increased and myofibrillar fibers were decreased after the injection of resistance hormone. Bing et al. (2006) studied the role of collagen fiber and its impact on the heart, indicating that myocardial collagen fiber has the role of connection and support, affects the transmission of information and the transport of nutrients and is also related to the contraction of the heart. Collagen fibers and myofibrils in myocardial cells can be distinguished by masson staining. The present study results showed that the muscle fibers of normal mice were arranged regularly and myofibrils were significantly more than collagen fibers; however, after the injection of resistin in mice, collagen fibers increased and myofibrils decreased. It is speculated that changes in the content of myofibrils and collagen fibers may, to a certain extent, affect the ability of the heart to stimulate and contract, the transmission of information and the transport of nutrients.

Fig 4: Effect of resistin on masson staining results of myocardium in mice.

Note: Fig A, C and E are masson staining results of control groups myocardium; Fig B, D and F are masson staining results of experimental groups myocardium.

Fig 5: Effects of resistin on the ultrastructure of myocardial cells in mice.

Note: Fig A, C, E are the ultrastructure of control groups myocardium; Fig B, D and F are the ultrastructure of experimental groups myocardium.
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Effect of resistin on ultrastructure of mice heart tissue

In the control group (Fig 5A, Fig 5C, Fig 5E), cardiomyocytes were arranged in a regular manner and each band was clearly visible. Cardiomyocytes in the control group exhibited a relatively intact plasma membrane, neatly arranged linear mitochondria, clearly visible transverse tubules and the interstitial tissue was not significantly changed. The mice in the test group (Fig 5B, Fig 5D and Fig 5F) had disorganized cardiomyocyte arrangement, unclear myofilament texture, less clear transverse tubules than in the control group, reduced number of mitochondria and mild lysis and blurring of mitochondrial cristae. The research shows that the ultrastructure of myocardium in diabetes rats changes: mitochondria proliferate, heterotopia, crowding, the double membrane gap of intercalated disc expands in a pool shape or a bag shape, myofilaments break off and dissolve and the capillary basement membrane is significantly thicker than that in normal rats (Weiwei et al., 2009). Mouse resistin resists insulin action and contributes to diabetes mellitus, while human resistin plays a role in inflammation and also functions as a small accessory chaperone (Tripathi et al., 2020). The results of this experiment showed that after the injection of resistin in mice, the myocardial cells of mice were disorderly arranged, the number of mitochondria in the sarcoplasm decreased and the mitochondria cristae were slightly dissolved and blurred. Mitochondria are the places where some eukaryotic cells generate energy and aerobic respiration. When the mitochondria change, the normal metabolic function of myocardial cells will be affected and the energy conversion will be affected. According to these structural changes, it is speculated that resistin may have some effect on cardiac physiological function.

CONCLUSION

The present study indicated that resistin result in thickening of myocardial cell membrane, arrangement of muscle fibers disorderly and increase of collagen fiber content; Also resistin make cytoplasmic loosely, blank areas in the cytoplasm appear and adjacent cell gaps increase. In a word, resistin may induce cardiac hypertrophy of mice. After injection resistin, the ultrastructure of myocardium has also undergone changes: the mitochondrial number decreases and mitochondrial ridges blur or dissolve slightly.

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