Deficiency of interfibrillar mitochondria in post-acute myocardial infarction heart failure

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Abstract: Mitochondrial dysfunction plays an important role in the progress of heart failure (HF). A pronounced variability of defects in mitochondrial subpopulations is reported to occur in various disease models. The aim of the study was to define the defects in the ultra structure and bioenergetic function of cardiac mitochondria in acute myocardial infarction-induced HF. AMI-induced HF rats were treated with saline (4.0ml/kg) for 8weeks. The ultra structure of myocardial mitochondrial subpopulations was assessed by electron microscope. The bioenergetic function of myocardial mitochondrial subpopulations was evaluated through Clark oxygen electrode. Results indicated that myocardial mitochondrial subpopulations in Model group had abnormal mitochondrial morphology which manifested as swelling and vacuoles, membrane lysis, fuzzy ridge structure, cristae lysis or disappear in IFM particularly, while SSM was almost survived in AMI induced heart failure. Results showed that the oxidative phosphorylation function of respiratory chain of NADH oxidation was impaired notably. Compared with Sham group, both P/O ($P<0.01$) and OPR ($P<0.01$) of myocardial IFM in model rats decreased, and V3 ($P<0.01$), P/O ($P<0.05$) and OPR ($P<0.01$) of SSM in Model group decreased either. Meanwhile, the oxidative phosphorylation function of respiratory chain of FADH oxidation was injured in SSM particularly, which presented as the decreased P/O ($P<0.01$). We propose that the mitochondrial defect of severe HF mostly lies in the interfibrillar mitochondria rather than in the subsarcolemmal mitochondria.

Keywords: Heart failure, myocardial mitochondrial subpopulations, mitochondrial ultra structure, mitochondrial respiration.

INTRODUCTION

After the treatment of heart failure over the past decades, the mortality rate among patients with heart failure still remains about 20% over 2 years, highlighting the reality that heart failure is still a serious clinical and public health problem (Roger, 2013; Sacks *et al.*, 2014).

Myocardial energy metabolism disorder caused by mitochondrial dysfunction is the pathological basis of myocardial energy crisis in heart failure. Myocardial mitochondria research based on 21 patients with chronic heart failure (less than 60% ejection fraction, NYHA Class II) indicated that more than 90% of myocardial mitochondria had structural and functional changes, and the pathological changes of myocardial mitochondria appeared earlier than clinical symptoms (Guzman *et al.*, 2014), suggesting that mitochondrial dysfunction plays an important role in the progress of heart failure (HF)

There exist two mitochondrial subpopulations in myocardia, subsarcolemmal mitochondria (SSM), which are situated beneath the plasma membrane, and interfibrillar mitochondria (IFM), which are located among the myofibrils. Studies based on myocardial mitochondrial subpopulations in various disease models indicated that SSM and IFM showed heterogeneous structural and functional injuries (Courtney *et al*., 2010; Erinne *et al*., 2009; Erinne *et al*., 2013; José *et al*., 2009; Jung *et al*., 2003; Mariana *et al*., 2008; Palmer *et al*., 1977; Palmer *et al*., 1986; Tim *et al*., 2009). Most studies on myocardial mitochondria in HF were performed on the SSM population of cardiac mitochondria, which prepared by polytron homogenization and differential centrifugation. While the two myocardial mitochondrial subpopulations may be affected differently in HF. Therefore, the aim of the study was to define the defects in the ultra structure and bioenergetic function of cardiac mitochondrial subpopulations in acute myocardial infarction-induced HF.

MATERIALS AND METHODS

Reagents and materials

Bradford Protein Assay Kit (Beyotime Institute of Biotechnology, China); Mito Tracker Deep Red 633 (Invitrogen, USA); L-Glutamate, Succinic acid (AMRESCO, USA); L-Malic acid (Solarbio, China); Retenone (Tocris Bioscience, UK); SPSS20.0 statistical analysis software (IBM, USA); ADP and other reagents were purchased from Sigma (St. Louis, MO, USA) and were of the highest purity grade.

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Animal model

Twelve male SD rats weighing 250±20g were purchased from Beijing Vital River Experimental Animal Co., Ltd [License No: SCXK (Beijing), 2012-0001]. The rats were kept under a 12 hours cyclic lighting, temperature (24±2 °C) and humidity (45%-50%) controlled SPFII Animal Lab (Guang'anmen Hospital, China Academy of Chinese Medicine Sciences). All experimental procedures were approved by the Ethics Committee of Guang'anmen Hospital, China Academy of Chinese Medicine Sciences.

After preoperative fasting for 16-24h, all rats were equilibrated in weight and divided into Sham group and Model group using Excel random number table method randomly. The approach of post-AMI heart failure animal model reported by Pfeffer (Pfeffer *et al*, 1990) was referred to. Male SD rats were intraperitoneally injected for narcosis, and the left anterior descending branch of coronary artery was ligated to cause acute myocardial infarction. For preparation of the sham animal models, only about 2-3mm under the starting point of anterior descending branch of left coronary artery was pierced, where there was no ligation. Other steps were the same as the preparation method of post-AMI heart failure animal model. Gavage was performed with 4.0ml•kg-1 saline for 8 weeks, once a day.

Preparation of cardiac mitochondrial subpopulations

The left ventricular free wall was flushed 5-10 times using phosphate-buffered saline (PBS; pH 7.4), dried and weighed. SSM and IFM were prepared on ice and adjusted with Palmer's approach (Palmer *et al*, 1977). The protein content was determined with Bradford method. With bovine serum albumin (BSA) as a standard, the protein concentration was adjusted to 10-20mg/ml.

Ultra structure of rat cardiac mitochondrial subpopulations

After fixation, dehydration, saturation, embedding, sectioning, staining and other conventional electron microscopic treatments, rat cardiac mitochondrial subpopulations were observed under an H-7650 transmission electron microscope (TEM) and imaged.

Respiratory function of cardiac mitochondrial subpopulations

A proper amount of respiration-determining medium [containing (in mmol/l) 15 KH_2PO_4, 15 KCl, 50 Tris base, 225 sucrose, 5 $MgCl_2$ and 0.1 EDTA-2 K (pH7.4)] was added to the reaction vessel with a total volume of 1mL and incubated for 2 min at 30^oC, to make it air saturated, followed by addition of 1 mg mitochondrial suspension, the state was recorded (State I). State 2 rates were measured for 1-2 minutes by addition of 10μL 10mM L-Glutamate+5mM L-malate, or 10μL 10mM succinate in the presence of 5μL 0.005μg/ml rotenone. And after added 5μL 250μM ADP, State 3 rates (V3, nmol·min-1·mg-1 mitochondrial protein) were measured as the rate of oxygen reduction mimicked those of the State 2 rates; State 4 rates (V4, nmol·min-1·mg-1 mitochondrial protein) were measured after exhaustion of ADP. RCR, P/O and OPR were calculated by the following formula. RCR = V3/V4, that is, the respiratory rate ratio between V3 and V4. P/O = ADP (exhausted ADP)/ oxygen atoms exhausted in State III (reduced oxygen concentration× vessel volume×2). OPR=V3×(P/O) [nmol·min-1·mg-1(protein)].

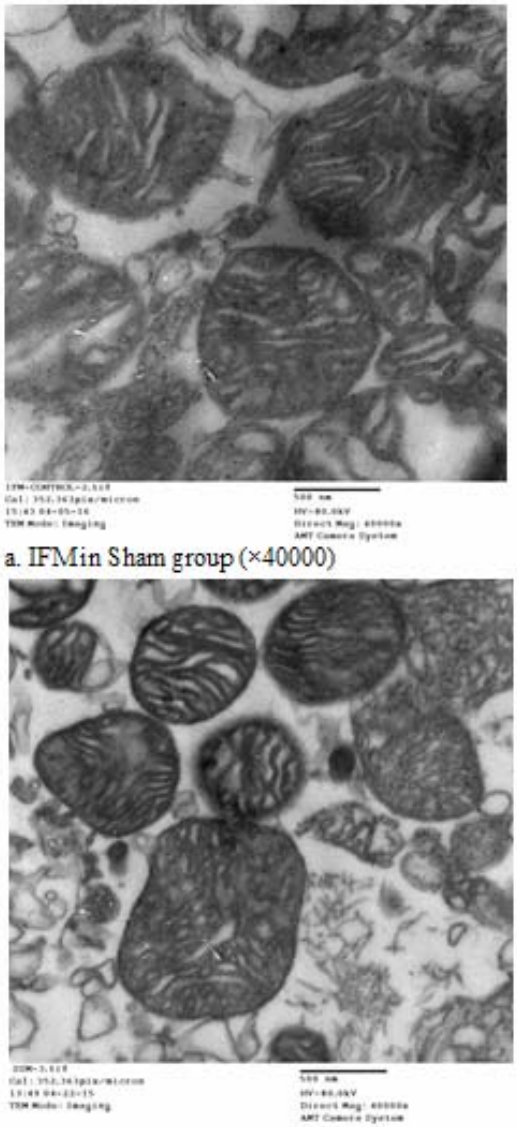

Fig. 1: The ultrastructure TEM images of cardiac mitochondrial subpopulations in Sham group.

STATISTICAL ANALYSIS

SPSS20.0 statistical analysis software was used for statistical analysis. For data that conformed to a normal distribution, two independent samples t-test was adopted for intergroup comparison. The data were represented by

mean ± standard errors. For data that didn't conform to a normal distribution, non-parametric test was adopted. A value of $P<0.05$ were considered to be statistically significant.

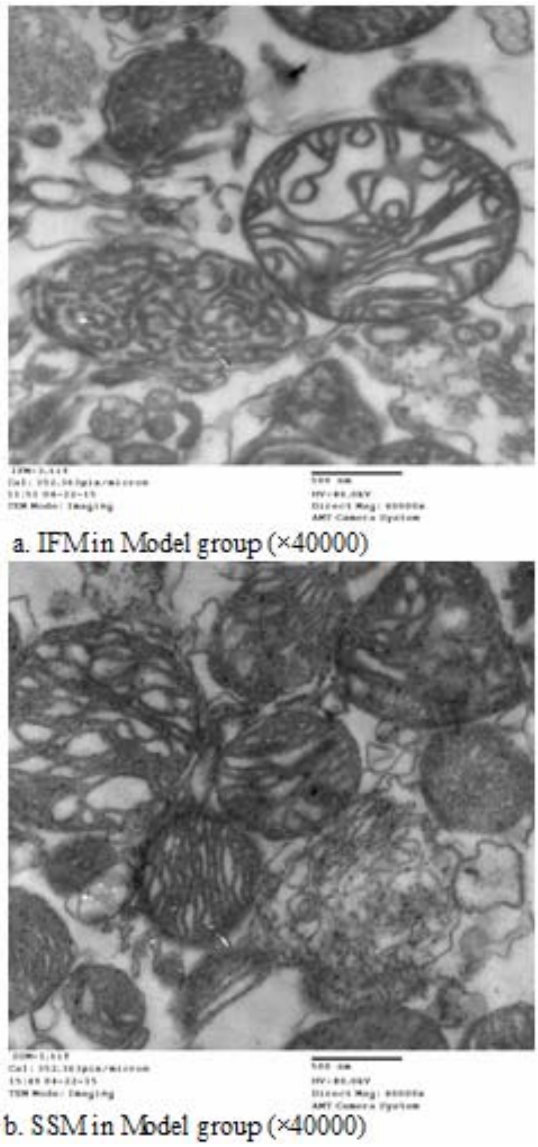

Fig. 2: The ultra structure TEM images of cardiac mitochondrial subpopulations in Model group.

RESULTS

Effect of heart failure on the ultrastructure of myocardial mitochondrial subpopulations

Ultra structural changes of cardiac mitochondrial subpopulations in heart failure

The ultra structure of the myocardial mitochondrial subpopulations in each group was observed under the transmission electron microscope. Results indicated that the mitochondrial subpopulations in Sham group had regular morphology. The size of IFM was in order, and the mitochondria were closely arranged. SSM did not have uniform size, and the mitochondria were loosely arranged, with less contact between mitochondria than that of IFM (fig. 1). The heterogeneity was found between IFM and SSM in Model group, which manifested as follows: the size of IFM was increased in Model group, and swelling outer chamber accompanied with swollen inner chamber could be seen; in addition, the matrix electron density was reduced, multiple focal vacuoles could be seen, the shortened crests moved around and reduced in number, the contact between mitochondria weakened, and the mitochondria were arranged looser than those in Sham group. While the size of SSM in Model group showed no obvious changes, which only manifested as enhanced contact between mitochondria, and the mitochondria were arranged more compact than those in Sham group (fig. 2).

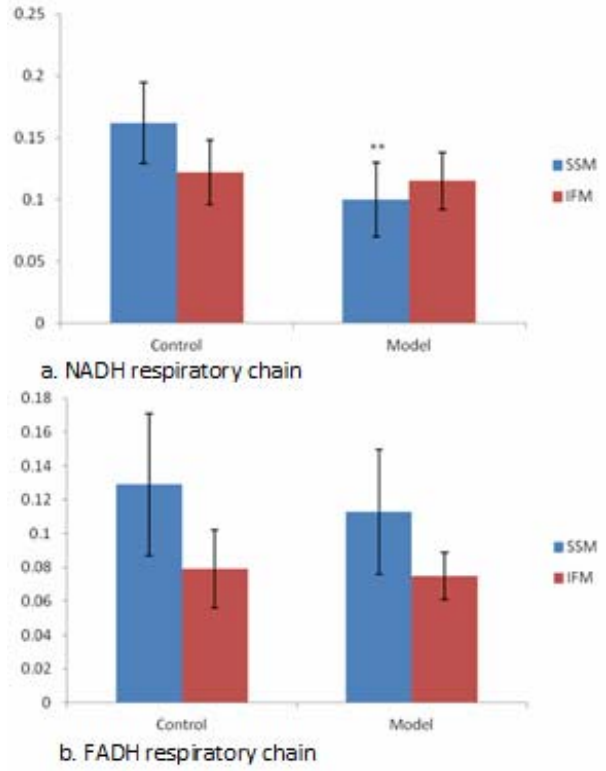

Fig. 3: The results of V3 from rat cardiac mitochondrial subpopulations in heart failure.

Structural changes in the cristae of cardiac mitochondrial subpopulations in heart failure

Various degrees of crest structural changes had been shown in the myocardial mitochondrial subpopulations of rats in the two groups. Crests in IFM contained the lamellar and tubuli-vesicular structures, while those of the SSM subpopulation mitochondrial crests were mostly lamelliform, which was consistent with the results of previous observation (Riva *et al*, 2005) (fig. 1). The crest morphology of IFM in Model group displayed multiple pathological changes, while the SSM showed no obvious changes. The mitochondrial crests of IFM in Model group

were disordered arranged and fused together and there were ruptures in the connecting parts of the crests (fig. 2).

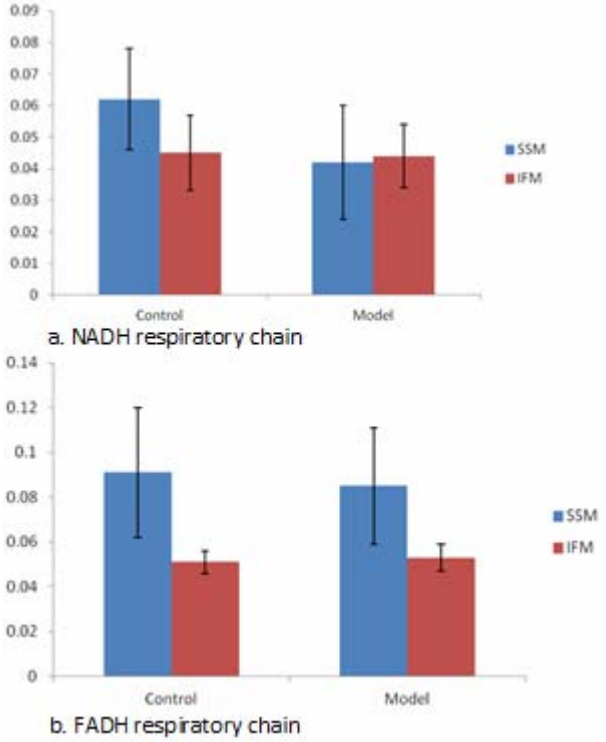

Fig. 4: The results of V4 from rat cardiac mitochondrial subpopulations in heart failure

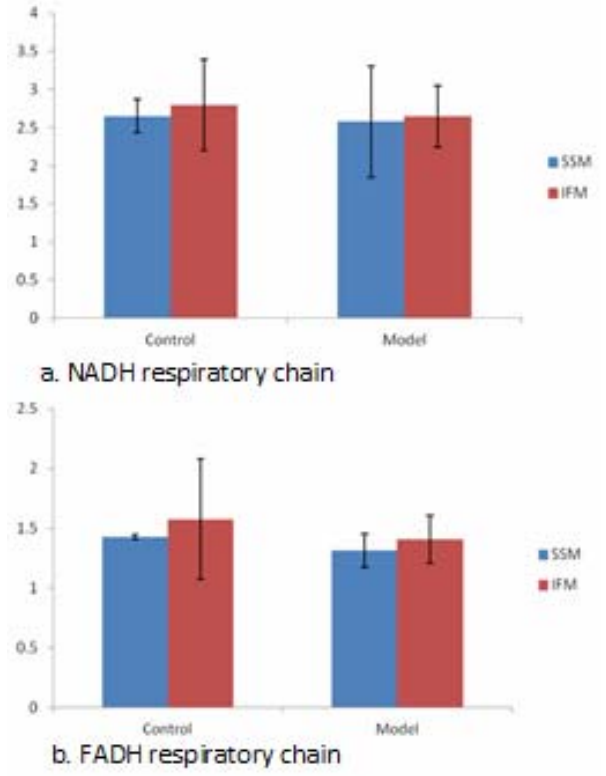

Fig. 5: The results of RCR from rat cardiac mitochondrial subpopulations in heart failure.

Effect of heart failure on the respiratory function of myocardial mitochondrial subpopulations

Functional changes in NADH oxidation respiratory chain

With L-Glutamate and L-Malic acid as the substrate, the oxidative phosphorylation function of NADH oxidation respiratory chain was determined. Compared with Sham group, both P/O ($P<0.01$) and OPR ($P<0.01$) of IFM decreased in Model group and the difference was significant. The V3 ($P<0.01$), P/O ($P<0.05$) and OPR ($P<0.01$) of SSM in Model group were significantly decreased (fig. 3a-fig. 7a).

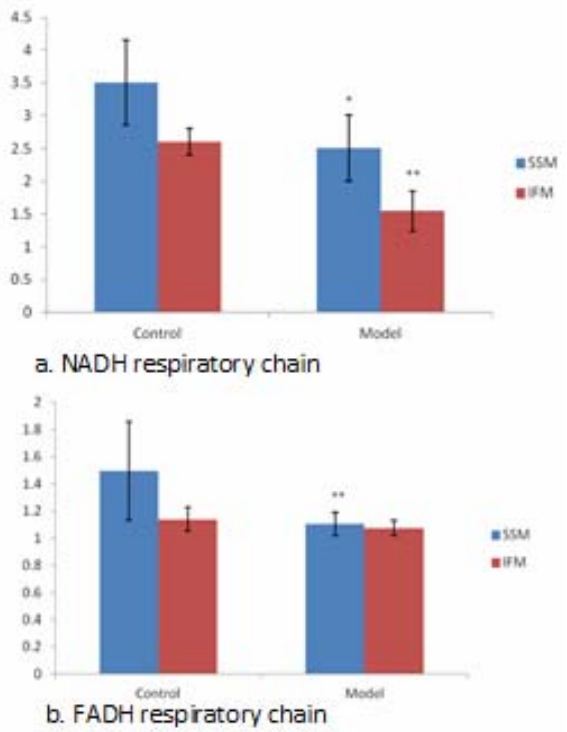

Fig. 6: The results of P/O from rat cardiac mitochondrial subpopulations in heart failure.

Functional changes in FADH oxidation respiratory chain

With succinic acid as the substrate, and the electron transfer of NADH→CoQ was inhibited with rotenone, the oxidative phosphorylation of FADH oxidation respiratory chain was determined. Compared with Sham group, P/O ($P<0.01$) of SSM in Model group was reduced and the difference was significant (fig. 3b, fig.7b).

DISCUSSION

Mitochondria, as a power switching station of myocardia, play an important role in maintaining the diastolic and systolic functions of myocardia. The electrons are passed down gradually through the inner membrane electron transport chain (ETC), and the potential energy obtained maintains the gradient of hydrogen ions of the inner membrane and drives the ADP to translate into ATP. When heart failure happened, under the sustained action

of ischemia, hypoxia, calcium overload and oxidative stress, the permeability of mitochondrial membrane increased, led to the enhance of mitochondrial colloid osmotic pressure, which caused mitochondria swelling, accompanied with matrix edema. The structure of mitochondrial inner membrane was damaged, which decreased the efficiency of electron transfer in inner membrane and decoupled oxidative phosphorylation. This cascade of reactions eventually leading to disorder in ATP synthesis and myocardial "energy starvation". And due to the ineffective electron transfer between electron acceptors, electron leakage increased, leading to the excessive generation of ROS and per oxidation damages to membrane protein, cardiolipin and mtDNA, exacerbating the disorder of cardiac mitochondrial oxidative phosphorylation and forming a vicious cycle of ROS-mediated ROS generation. Excessive generation of ROS can raise the sensitivity of Ca2 + -induced PTP opening. On the pathological basis of calcium overload in myocardial cells, the open state of mPTP was changed from a transient low conductance mode to a continuous high conductance pathological opening mode, causing irreversible damages to the shape of mitochondria, and down-regulating membrane potential, further inhibiting the oxidative phosphorylation function of mitochondria and formed a pathological cascade cycle (Halestrap *et al*., 2009; Weiss *et al*., 2016)

Results indicated that myocardial mitochondrial subpopulations in Model group had abnormal mitochondrial morphology which manifested as swelling and vacuoles, membrane lysis, fuzzy ridge structure, cristae lysis or disappear in IFM particularly, while SSM was almost survived in AMI induced heart failure. The oxidative phosphorylation of rat myocardial mitochondria in the post-acute infarction heart failure model was significantly damaged, especially the damages to NADPH oxidation respiratory chain was notable, characterized by the pathological change of reduced ATP synthesis efficiency. This was consistent with the results in previous studies (Toga *et al*., 2007; Marunouchi *et al*., 2013), which also proved that the damages to NADPH oxidation respiratory chain in IFM subpopulation were especially significant. Previous studies suggested that compared with SSM, the oxidative respiratory capacity of IFM was 1.5 times higher than that of SSM (Palmer *et al*., 1977), which was contradictive to this study, as the oxidative respiratory capacity of SSM was greater than that of IFM. The experimental animal applied by Palmer *et al*. was male SD rats weighing 250-300g, about 6 weeks old. While male SD rats adopted in this study were about 18 weeks old, and the average weight was 700-750g. Researches had showed that the decline of enzymatic activity related to oxidative phosphorylation was proportional to the degree of aging (Erinne *et al*., 2013; Jung *et al*., 2003; Mariana *et al*., 2008; Tim *et al*., 2009). Based on the above discussion, it could be considered that the disparate results were caused by different ages of animals adopted to prepare myocardial mitochondrial subpopulations.

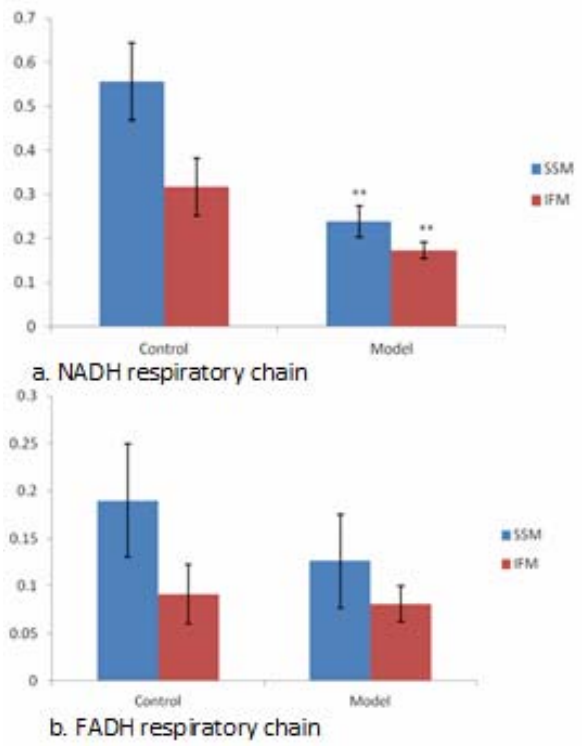

Fig. 7: The results of OPR from rat cardiac mitochondrial subpopulations in heart failure.

In summary, the ultra structural damage and oxidative phosphorylation dysfunction of rat myocardial mitochondria in the post-acute infarction heart failure model mostly occurred in IFM subpopulation. Combined with the heterogeneous biological distribution and function between IFM and SSM, i.e., IFM was adjacent to myofibril, and compared with SSM, the internal structure of IFM was compacter and more regular, characterized by higher membrane potential and oxidative phosphorylation coupling. Therefore, the principal biological function of IFM was to provide sufficient ATP energy for the systolic mechanism of myocardia. The biochemical dysfunction of IFM had more direct correlation with the diastolic and systolic dysfunction of heart failure.

CONCLUSION

We propose that the mitochondrial ultra structure and respiration defects of severe HF mostly lies in the interfibrillar mitochondria rather than in the subsarcolemmal mitochondria. Furthermore, ultra structural and biological studies of myocardial mitochondrial subpopulations can help us master the heterogeneous pathological changes of IFM and SSM in heart failure and provide a favorable direction for going deep into myocardial mitochondria relevant pathological mechanisms in heart failure.

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