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Exercise training-induced different improvement profile of endothelial progenitor cells function in mice with or without myocardial infarction

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ABSTRACT

Background: Neovascularization in response to ischemia after myocardial infarction (MI) has been widely considered as being initiated by endothelial progenitor cells (EPCs). Well-documented evidences in recent years have proved exercise training (ET) improving EPC function. However, whether ET-induced improvement of EPC function under or without ischemic state is different has not been reported.
Methods: Mice performed ET following an exercise prescription 1 week after MI or non-MI surgery respectively. Bone marrow-derived EPCs were isolated at 0 day, 3 days, 1 week, 2 weeks, 4 weeks, and 8 weeks of ET. After 7 days cultivation, EPC functions including proliferation, adhesion, migration, and *in vitro* angiogenesis were measured. AKT/glycogen synthase kinase 3β (GSK3β) signaling pathway was tested by western blotting.
Results: EPC function in mice underwent non-MI surgery was attenuated overtime, while ET ameliorated this tendency. EPC function was peaked at 4 weeks ET in non-MI surgery mice and maintained with an extended exercise time. Besides, simple ischemia was sufficient to enhanced EPC function, with a maximum at 2 weeks of MI surgery. In MI mice, ET further improved EPC function and achieved peak at 2 weeks exercise. Furthermore, AKT/GSK3β signaling pathway activation was consistent with EPC function change after ischemia, which was further promoted by 4 weeks exercise.
Conclusion: ET significantly increased EPC function in mice both with and without MI, but the time points of peak function were different.

1. Introduction

Postnatal vasculogenesis and reendothelialization induced by endothelial progenitor cells (EPCs) that in turn stimulated by ischemia was reported as a potent intervention after myocardial infarction (MI) [1]. Physical exercise was widely proved against cardiovascular diseases, including MI. To date, aerobic exercise-based cardiac rehabilitation has been listed as a first-line therapeutic approach recommended in many guidelines to reduce mortality after acute MI, such as the European Society of Cardiology (ESC) Guidelines for the management of acute MI in patients presenting with ST-segment elevation, and in the ESC guidelines on myocardial revascularization [2,3].

It has been reported that therapeutic potential of EPCs could be improved markedly by exercise training (ET) [4]. In recent years, emerging studies have explored the relationship between ET and EPCs, but some questions remain to be elucidated. For example, how does ET affect EPC function? Whether the effects of short-term and long-term ET on EPC function are different? Furthermore, whether ET induced EPC function achieving peak time points under or without ischemic status is different? Based on those unsolved questions, we designed this study in mice.

Protein kinase B (PKB/AKT)/glycogen synthase kinase 3β (GSK3β) signaling pathway was identified involving in cardioprotective actions after myocardial ischemia [5,6]. Previous study found exercise exerting its protective role *via* activation of AKT/GSK3β signaling pathway [7]. More interestingly, some EPC stimulator was also reported to associate with this cascade activation [8]. Thus, we tested the activation of AKT/GSK3β pathway to explore the mechanism of exercise induced EPC function after MI or without MI.

2. Materials and methods

2.1. Myocardial infarction model

Male Kunming mice (body weight of 28 to 32 g, aging of 6 weeks) were obtained from Medical Experimental Animal Center of Hunan Province, China. MI model was constructed by ligating the left anterior descending coronary artery as previously described [9]. The non-MI (sham operation) mice underwent the same surgical procedure but without ligating left anterior descending coronary artery. The protocol was approved by the Animal Research Committee, Central South University, Hunan, China.

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2.2. Exercise training protocol

Mice post-surgery had 7 days rest. The survived mice were randomly divided into four groups: non-MI and non-ET (NMI NET); non-MI with ET (NMI ET); MI and non-ET (MI NET); MI with ET (MI ET) (n = 14 each in five independent experiments respectively. The following survival rate was over 90% and 12 mice in each group were needed). The ET groups were exercised following a progressively increased workload protocol [10] and control groups were fed in the same conditions. Exercise protocol began with 10 min at a speed of 10 m per minute; and added 10 min per day with a speed increased 1 m per minute. After 5 days exercise, all mice had 2 days rest. From the second week, exercise mice underwent ET following a protocol of 1 h at a speed of 15 m per minute and exercised 5 days per week.

2.3. Bone marrow-derived EPC isolation, culture and identification

EPCs were isolated by density gradient centrifugation at 0 day, 3 days, 1 week, 2 weeks, 4 weeks and 8 weeks of ET. Every 2 mice (selected by a random digit table) in four groups were killed by excessive anesthesia in accordance with rules of the Institutional Ethical Board for Experimental Procedures. The mouse tibiae and femurs were blunt dissected. Then the medullar channels were flushed with 5 mL phosphate buffered saline (PBS). Medullar rinses were suspended on 3 mL HISTOPAQUE1083 (Sigma) and centrifuged at 400 *g* for 30 min. The cloudiness layer of cells was aspirated out and mixed with 3 mL Dulbecco's Modified Eagle's Medium (DMEM).

After centrifuged at 400 *g* for 10 min, supernatant was removed and cells were resuspended with endothelial cell basal medium-2 (EBM-2) with 20% fetal bovine serum (FBS; Gibco) supplemented and added vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), ascorbic acid, hydrocortisone, heparin and antibiotics, according to the manufacturer's instructions (Singlequots, Lonza). EPCs were isolated by subsequent purification over Ficoll gradients and cultured at a density of $5 \times 10^5/cm^2$. Cells were planted on 25 cm^2 cell culture bottle coated with human plasma fibronectin purified protein (10 μg/mL; Millpore). After 4 days culture, non-adherent cells were removed. Adherent cells were cultured for another 3 days. The same process in the control group and groups at all ET time points was repeated; each group was independently performed five times.

After 7 days culture, EPC expression of antigens CD34, CD133, CD31 and VEGFR2 were detected by flow cytometry. Relative isotype controls were used as negative controls.

2.4. Proliferative ability assay

EPCs calculated 1×10^4 per well were seeded on the plate coated with human fibronectin purified protein and cultured for 24 h. Then 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (Sigma) per well were supplemented and incubated avoid light for another 4 h. Supernatant medium was aspirated remaining 50 μL per well and each well added 150 μL dimethylsulfoxide (DMSO). After 10 min shaken, optical density (OD) value was measured at 490 nm light absorbance.

2.5. Adhesive ability assay

EPCs calculated 1×10^5 per well were seeded on the plate coated with human plasma fibronectin purified protein and incubated for 60 min at 37 °C. Non-adherent cells were aspirated and rinsed 3 times with PBS gently. Results were the mean number of adherent cells counted under microscope (magnification ×200) from five random fields.

2.6. Migratory ability assay

EPCs calculated 3×10^5 per well were transferred into the upper chambers of transwell plate (Millipore). The lower chambers were loaded with EBM-2 medium with more 50 ng/mL VEGF (Peprotech) added to promote migration. After 12 h migration, migrated cells on outside of the membrane were stained with hematoxylin and eosin (H&E). Results were the mean number of migrated cells counted under microscope (magnification ×200) from five random fields.

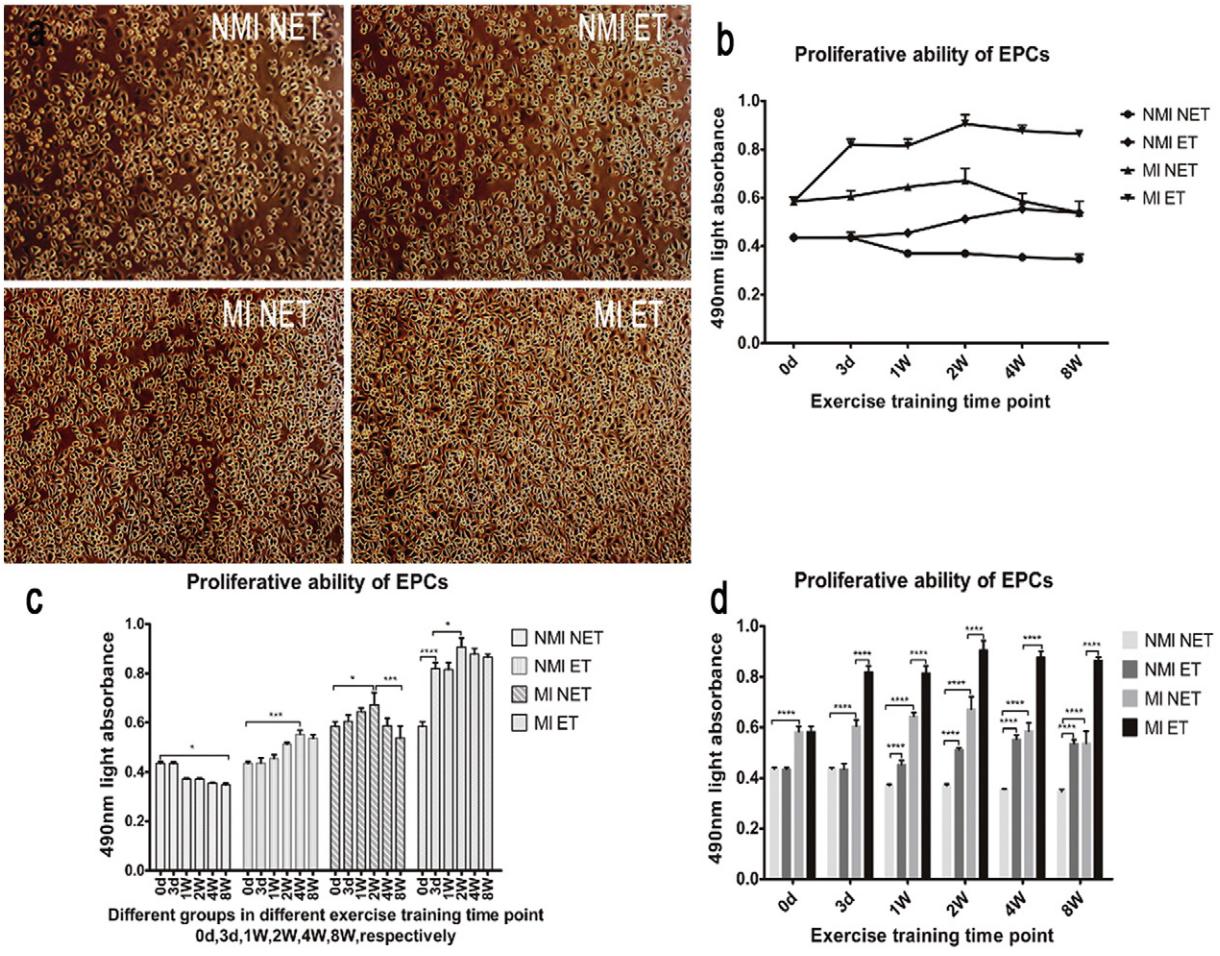

Fig. 1. ET improves EPC proliferative ability. After 7 days culture, EPCs were resuspended and proliferative ability was measured by MTT method. The OD values of EPCs were measured under 490 nm light absorbance. a. Effects of ET for 4 weeks on EPC proliferative ability after 7 days culture. b. The OD value variation trends at different ET time points. c. Comparison of OD value at different ET time points. d. Comparison of OD value at different groups. NMI NET: non-myocardial infarction and non-exercise training; NMI ET: non-myocardial infarction with exercise training; MI NET: myocardial infarction and non-exercise training; MI ET: myocardial infarction with exercise training. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.