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添付資料：研究報告書

中国人研究者名：李 予 昕

指導責任者名：福田 昇 職名：助教授

所属機関名：日本大学医学部

〒173－0031

所在地：東京都板橋区大谷口上町30－1

電話：03－3972－8111 内線：2717

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2．研究テーマ

心筋幹細胞寿命延長による心不全の保存的再生医療

3．成果の概要（100字程度）

1 心筋細胞分離方法の確立: Langendorff法 心臓灌流、単純心筋細胞分離

2 心筋幹細胞の同定：免疫染色方法で幹細胞マーカーの発現

3 心筋幹細胞の機能：心筋幹細胞は年齢とともに数は少なくなる

4 スタチンの心不全モデルマウスでの心筋幹細胞寿命への作用：実行中

4．研究業績

(1) 学会における発表 (無) ・ 有（学会名・演題）

(2) 発表した論文 (無) ・ 有（雑誌名・題名）

－日中医学協会助成事業－

心筋幹細胞寿命延長による心不全の保存的再生医療

研究者氏名　李　予昕

中国所属機関　阜外心血管病医院超音波科

日本研究機関　日本大学医学部循環器内科

指導責任者　助教授　福田　昇

Abstract

Cardiac myocytes have been traditionally regarded as terminally differentiated cells that adapt to increased work and compensate for disease exclusively through hypertrophy. However, in the past few years, compelling evidence has accumulated to suggest that the heart tissue has regenerative potential. Discovery the cardiac stem cell certainly opens new opportunities for myocardial repairing, and conservative medicine to prolong life span of cardiac stem cell will be useful as new regenerative medicine for chronic heart failure. Present study examined effects of statin on senescence and function of cardiac stem cell in mice heart failure models. Wild type and Adrenomedullin knockout C57BL/6 male mice at 6 weeks age were used. Heart failure model was made by 12 weeks continuous high-salt diet. Five mg/kg/day atorvastatin was continuously oral administrated in both wild type and knockout mice. Cardiac stem cells were identified by immunofluorescence, fluorescence activated cell sorting (FACS) and real time PCR. Cardiac stem cell function was estimated with oxidative stress, cellular senescence and tolemere related proteins. Although percentage was very low, cardiac stem cells were identified by immunofluorescence with anti C-Kit antibody. The number and function of cardiac stem cell decreased as the mice age increased.

Background

Implantation of bone marrow (BM)-derived stem cells or other stem cells will be useful to improved failure organs which can not be improved present medicines. Implantation of mesenchymal stem cells into myocardium has recently been tried to improve the cardiac function in patients with severe heart failure, which transiently improved the impaired cardiac function. However, since the heart failure is a chronic disease, the transient improvement of cardiac function does not sufficiently prolong the life span in patients with the chronic heart failure.

Cardiac myocytes have been traditionally regarded as terminally differentiated cells that adapt to increased work and compensate for disease exclusively through hypertrophy. However, in the past few years, compelling evidence has accumulated to suggest that the heart tissue has regenerative potential. The adult heart has been confirmed to contain undifferentiated cells with the characteristics of cardiac stem cells. These cells have been isolated and expanded from adult heat (human, mice, rats) [1,2]. Characteristics of the cardiac stem cells are self-renewing, clonogenic, and multipotent. They differentiate three cell types: myocytes, smooth muscle, and endothelial vascular cells. Moreover, when injected into an ischemic heart, a population of these cells or the clonal progeny of one of them reconstitute a well-differentiated myocardial wall that encompasses up to 70% of the left ventricle. Discovery the cardiac stem cells certainly opens new opportunities for myocardial repairing.

Stem cells that exist to attach the niche in bone marrow are derived to peripheral blood after separation from the niche and act to repair the tissue damage. Stem cells are easily damaged with oxidative stress that shorten their cell cycle as the stem cell senescence. Cardiac stem cells show different life span in wild-type mice and insulin-like growth factor-1 transgenic homozygous mice [3].

Thus conservative medicine to prolong life span of cardiac stem cell will be useful as new regenerative medicine for chronic heart failure.

Methods

Animal

C57BL/6 male mice at 6 weeks age (AM+/+, wild type) and Adrenomedullin knockout C57BL/6 male mice at 6 weeks age (AM+/-) were used in the present study.

Heart failure model and statin administration

Heart failure model was made by 12 weeks continuous high-salt diet (8%) and confirmed by echocardiography. Five mg/kg/day atorvastatin was continuously oral administrated in both wild type and knockout mice.

Cardiac myocyte isolation

A heart of adult mouse was enzymatically dissociated into a single cell suspension as described previously [4,5]. Briefly, hearts were attached to a Langendorff perfusion system and perfused with 0mmol/L Ca2+ solution consisting of (mmol/L) 126 NaCl, 4.4 KCl, 1.0 MgCl2, 13 NaOH, 24 HEPES, 2.5 g/L taurine, 0.65 g/L creatine monophosphate, 0.55 g/L sodium pyruvate, 0.14g/L NaH2PO4, and 2 g/L glucose. Then the hearts were subsequently digested with 100μmol/L Ca2+ solution and washed with 100μmol/L Ca2+ solution without the enzymes. Both ventricles were excised, minced and shaken gently in 100μmol/L Ca2+ solution. The cell suspension was filtered though a fine metallic tea filter and isolated myocytes were kept in 1mmol/L Ca2+ solution at room temperature, and were used within 6h after the isolation procedure.

Cardiac stem cell identification

Cardiac stem cells were identified by immunofluorescence, fluorescence activated cell sorting (FACS) and real time PCR. Cardiac stem cells were marked by anti-C-Kit antibody, anti-Scr-1 antibody and anti-Isl-1 antibody.

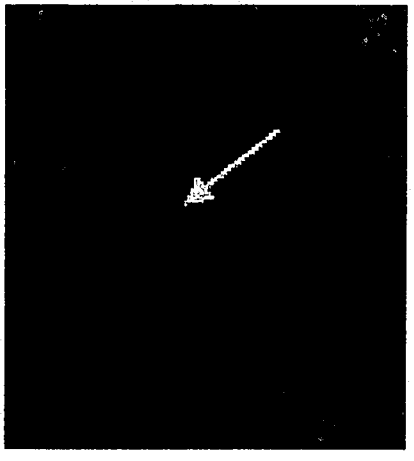
Estimate cardiac stem cell function

Oxidative stress was estimated with immunohistochemistry by anti 8-hydroxy-2'-deoxyguanosine (8-OH-dG) monoclonal antibody. Cellular senescence was estimated with p21Cip1, p27Kip1 and tolemere related proteins: TERT, phospho-Akt and total Akt.

Results

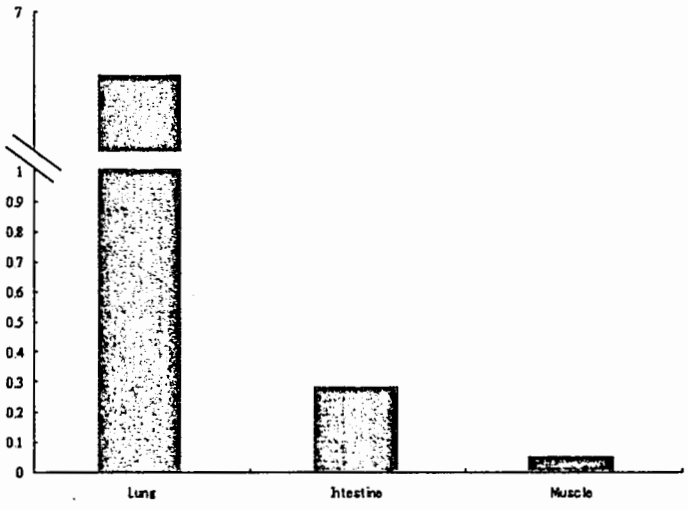
Cardiac stem cell identification

Cardiac stem cells were found by immunofluorescence with anti C-Kit antibody. The percentage of cardiac stem cell was very low, the nucleus of the cardiac stem cells were smaller than the nucleus of general cardiomyocytes (Figure 1).

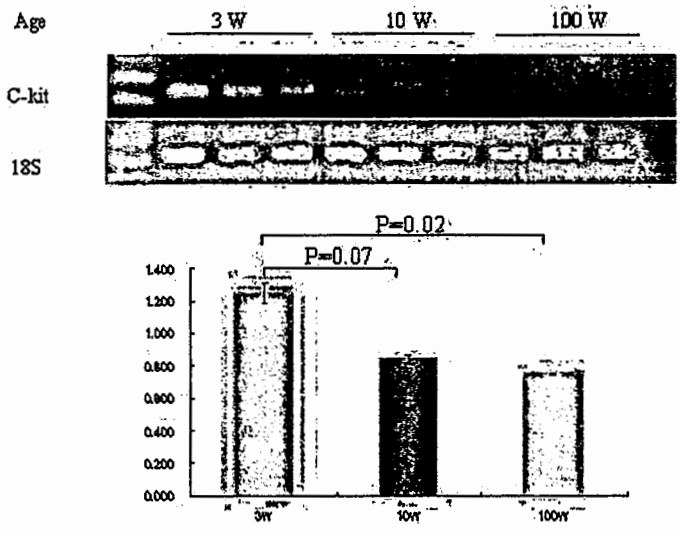


Cardiac stem cell function

Variant number of stem cells was identified in different tissues or organs. We measured C-Kit expression in lung, intestine and cardiomyocyte with real time RT-PCR in same mouse and found the C-Kit expression in lung was most and the expression in cardiomyocyte was least (Figure 2).



We measured C-Kit expression in cardiomyocyte with different age by real time RT-PCR. As representing in Figure 3, the expression of C-Kit decreased along with age increasing.



Discussion

Implantation of mesenchymal stem cells into myocardium has recently been tried to improve the cardiac function in patient with heart failure, which transiently improved the impaired cardiac function. However, since the heart failure is a chronic disease, the transient improvement of cardiac function is not enough to normalize the life span in patients with the chronic heart failure.

Cardiac stem cells were discovered to support myocardial regeneration [1,6,7]. They are self-renewing, clonogenic, and multipotent, giving rise to a minimum of three differentiated cell types: myocytes, smooth muscle, and endothelial vascular cells. Conservative medicine to prolong life span of cardiac stem cell will be therefore one of useful regenerative medicine for chronic heart failure.

From the preliminary results in the present study, we identified cardiac stem cells in adult mice heart. The cardiac stem cells percentage was very low. This result agreed with previous studies which proved the frequency of cardiac stem cell was 1~30 / 10^4 cardiomyocytes [1,4,8-10]. The nucleus of the cardiac stem cells was smaller than the nucleus of general cardiomyocytes. The number and function of cardiac stem cells decreased along with age increasing.

This study is in the process, we did not get the last results yet.

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