行政院國家科學委員會專題研究計畫　期中進度報告

高血脂症是否會誘發心肌肥厚及其致病機轉(1/3)

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執行單位： 國立臺灣大學醫學院一般醫學科

計畫主持人： 吳造中

計畫參與人員： 蘇銘嘉、楊怡凡

報告類型： 精簡報告

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行政院國家科學委員會補助專題研究計畫 □成果報告 ☑期中進度報告

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成果報告類型(依經費核定清單規定繳交)：☑精簡報告 □完整報告

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查詢

執行單位：

中　華　民　國　92　年　5　月　31　日

一、中文摘要：

依照本研究計畫的第一年目標，我們建立了新生小鼠的心臟細胞模型，並將利用此模型進行心臟肥厚機制的研究工作。此外我們也自健康的人血液中純化萃取低密度脂蛋白（LDL），經銅離子催化氧化後成為氧化態低密度脂蛋白（Oxidized LDL），用以瞭解氧化態低密度脂蛋白在冠狀動脈疾病發生時對心臟細胞直接的作用以及其作用機制。另外，TNF-α引發血管內皮細胞表現 ICAM-1 細胞黏著分子的細胞信息傳遞機制研究也在進行之中。

二、英文摘要：

As the planning goal of this first year's project, we established the research model of neonatal rat cardiomyocytes for studying the mechanisms of cardiac hypertrophy. In addition, we also purified low-density lipoprotein (LDL) from healthy donors' blood. We oxidized the LDL under the catalysis of copper ion and attempted to realize the acting mechanisms of oxidized LDL on cardiac myocytes when coronary artery disease occurred. Meanwhile, the signal transductional mechanisms of TNF-α induced ICAM-1 expression on endothelial cells were also under study.

三、報告內容：

I. 前言：

Cardiac hypertrophy is a common symptom when cardiac disease occurred, it also has been considered as a spontaneous adaptive response to such stress. Many studies suggested that the initial cardiac hypertrophic response was a kind of compensatory mechanism that was used to increase cardiac output ability via cardiac remodeling.[i,ii] However, sustaining stimulation of cardiac hypertrophy, finally will decompensate the heart and let it stepping toward the progression of hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) accompany with heart failure and sudden death.[iii] The key point that makes cardiac hypertrophy stepping toward failure was now still poorly understood. Recently, the endothelium is of great interest because its damages lead to a variety of diseases. Previous studies have shown that arterial endothelium provides a relative barrier to the uptake and transport of substances in the blood stream. As we know, changes in the permeability of arterial wall may cause alterations of the microenvironment surround blood vessel, and this alteration in cardiac tissue may induce a series of cellular effect and may exacerbate the progression of cardiovascular diseases. In our project, we attempted to carefully

investigate the mechanisms of cardiac myocytes damage in aspects of intracellular Ca^{2+} level regulation [iv, v, vi], cell apoptosis [vii] and the disintegrity of vascular endothelial cells[viii,ix].

II. 研究目的：

1. Setup the primary culture system of neonatal rat cardiomyocytes.
2. Isolation and culture of human umbilical vein endothelial cells (HUVEC).
3. Induction of hypertrophy in cardiomyocytes by neurohumonal factors or electrical stimulation.
4. Use confocal microscope to detect the immunostaining samples of cells.

III. 研究方法

1. Isolation and primary culture of cardiomyocytes. Heart ventricles isolated from 1–3-day-old newborn Wistar rats were minced, and received subsequent trypsin digestion steps. Isolated heart cells were suspended in a culture medium [Ham's F-12 medium supplemented with 5 μg/ml of insulin and transferrin] containing 10% fetal calf serum (FCS) and antibiotics (100 units/ml penicillin G and 100 μg/ml streptomycin). Non-cardiomyocytes were separated from the cardiomyocytes by differential preplating that the isolated heart cells were preincubated for 2.5 hr to separate nonadhering myocytes from adhering nonmyocytes. The purified myocytes (>95%) were suspended in the fresh culture medium containing 5-bromo-2'-deoxyuridine (BrdU; 100 mM) to inhibit proliferation of nonmyocytes, and seeded into collagen-coated culture plates. The plates were incubated in a 37^{o}C humidified 5% CO_2 incubator.

2. Immunostaining of cells and confocal microscopic examination. Plating cells were fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Then cells were blocked by 10% BSA followed by primary antibody treatment. A secondary fluorescein-conjugated antibody was used to detect the primary antibody. After mounting, samples were observed by laser confocal microscope under the control of computer system.

3. Oxidized LDL Preparation. Human LDL (d=1.019 to 1.063) was isolated by sequential ultracentrifugation from fresh plasma collected in 1 mg/ml EDTA. Moderately oxidized LDL was prepared by incubating 2 mg/ml LDL with 2 μM Cu^{2+} in EDTA-free PBS for 4 h at 37°C. Fully oxidized LDL was prepared by incubating 100 μg/ml LDL in medium with 10 μM Cu^{2+} for 24 h at 37°C. The degree of oxidation was estimated from thiobarbituric acid-reactive substances (TBARS)/mg of

protein by a fluorimetric method.

IV. 結果與討論：

Oxidized LDL induced cardiac myocytes apoptosis

There is an increasing body of evidence showing that oxidized LDL plays an important role in coronary artery disease. It also has been shown that oxidized LDL could cause apoptosis in different cells via various mechanisms. Here we have shown that oxidized LDL could induce cardiac myocytes apoptosis (Fig 1).

It has been long believed that apoptosis does not occur in terminally differentiated cells such as cardiomyocytes. However, studies in patients' hearts with dilated or ischemic cardiaomyopathy have demonstrated histological evidences of apoptosis. It has been proposed that the compensatory upregulation of gene transcraption during cardiac hypertrophy may fail these cardiac myocytes into apoptosis[x].

Does oxidized LDL directly lead cardiac myocytes to apoptosis via its receptors on the cells or through the upregulation of numbers of cell survival gene transcription and then failing the cardiac myocytes into death. Works are still undertaken to solve this question.

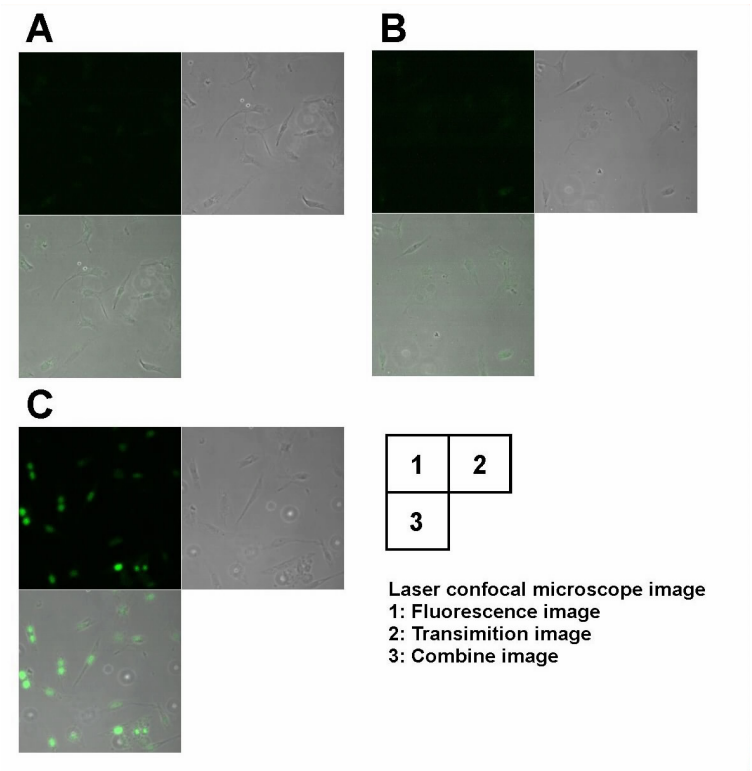

Fig 1. Oxidized LDL induced cardiac myocytes apoptosis. After 4% paraformaldehyde fixation, cells were treated with TUNEL reaction. Apoptosis cells were detected under laser confocal microscope. A, control group. B, cardiac myocytes were treated with 50nM $CuSO_4$ for 8hr. C, cardiac myocytes were treated with 17 μg/ml oxidized LDL (contain 50 nM $CuSO_4$) for 8hr.

Hypertrophic response of cardiomyocytes

To test the hypertrophic effect of cardiaomyocytes response to neurohormonal stimulations, cells were treated with 500ì M phenylapherine (PE) or 500ì M angiotensin II (Ang II) for 24 hr (Fig 2). Then cell area was quantified by computer software according to microscopic image. In our cell model, cell area could increase almost 30% after neurohormonal stimulation, and could provide us a screening tool for drug discovery.

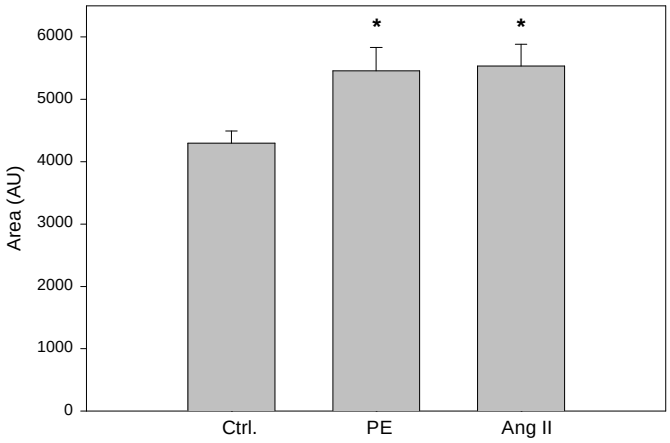

Fig 2. Hypertrophic response of cardiaomyocytes. Cells were treated with 500ì M PE or 500ì M Ang II for 24 hr. *P<0.001

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五、計畫成果自評：

In this first year's work, we spend much time on establishing and testing the cardiomyocyte culture model. For the future's tasks, we have got a promising goal to dissect the key point of cardiac hypertrophy and apoptosis process.