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(2/3)

計畫類別： 個別型計畫
計畫編號： NSC92-2314-B-002-220-
執行期間： 92 年 08 月 01 日至 93 年 07 月 31 日
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

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中　華　民　國 93 年 5 月 31 日

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

高脂血症是否會誘發心肌肥厚及其致病機轉

計畫類別：☑ 個別型計畫　　☐ 整合型計畫
計畫編號：NSC 91－2314－B002－283
執行期間：92 年 8 月 1 日至 93 年 7 月 31 日

計畫主持人：吳造中
共同主持人：蘇銘嘉
計畫參與人員：楊怡凡

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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☐出席國際學術會議心得報告及發表之論文各一份
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執行單位：一般醫學科

中　華　民　國　93　年　5　月　31　日

一、中文摘要：

本研究計畫執行的前兩年中，我們已建立起新生小鼠心臟細胞及人類臍帶血管內皮細胞培養的實驗模型，並將其應用於探討心血管疾病成因機制的研究。高脂血症是許多心臟血管疾病的重要因子，同時我們也注意到許多糖尿病患者常伴隨肥胖及高脂血症並併發心臟血管疾病，因此血糖濃度的變化亦是左右心臟血管疾病的重要因子之一。在我們的研究之中，我們將從細胞生物學的角度探討氧化態低密度脂蛋白（Oxidized LDL）及葡萄糖對血管內皮細胞及心臟肌肉細胞的影響。

二、英文摘要：

In the first two years of this project, we have established culture systems of neonatal rat cardiomyocytes and human umbilical vain endothelial cells. We have applied these culture systems into our research of cardiac and vascular diseases. Dyslipidemia is an important risk factor for the cardiovascular disease. Many of the diabetic patients have dyslipidemia and suffer from cardiovascular syndromes. Therefore, the change of blood glucose is also an important factor for cardiovascular diseases. In our study, we have investigated the effects of oxidized LDL and glucose on our cell culture system.

三、報告內容：

I. 前言：

In the third year of this project, we will continue the second year's work and also take another focus on the functional assay of cardiomyocytes. In cardiomyocyte, sacroplasmic reticulum (SR) takes the most important role in regulating intracellular Ca^{2+} level. By the advantages of confocal microscope, we can detect the Ca^{2+} changes in subcellular level, and measure the contraction of myocytes. The regulation of Ca^{2+} transient and the shortening of the cell may both represent the function of myocytes.

II. 研究目的：

1. Evaluate the cytotoxicity effect of oxidized LDL in various concentrations of glucose.
2. Setup systems for measuring intracellular Ca^{2+} concentration.

III. 文獻探討：

IV. 研究方法：

1. Measurement of intracellular [Ca^{2+}] with fura-2. [Ca^{2+}]i was measured as described previously.[i] After stabilization of the preparations, fura-2 potassium salt was microinjected iontophoretically into one cell and allowed to spread throughout the muscle via gap junctions. [Ca^{2+}]i was determined by measuring the epifluorescence of fura-2 in the cell, excited using ultraviolet light at 380 nm and 340 nm. The fluorescent light was collected at 510 nm by a photomultiplier tube. After equilibration of the loaded fura-2, intracellular free Ca^{2+} concentration and contractile force were measured. The outputs of the photomultiplier tube and force transducer were filtered at 100 Hz, collected by an A/D converter and stored in the computer for later analysis. Intracellular [Ca^{2+}] was given by the following equation (after

subtraction of the autofluorescence of the muscle):

$[Ca^{2+}]i = Kd\ (R - R\text{min})/(R\text{max} - R)$

where *R* is the observed ratio of fluorescence (340 nm/ 380 nm), *K*d is the apparent dissociation constant, *R* max is the ratio of 340 nm/380 nm at saturating $[Ca^{2+}]$, and *R*min is the ratio of 340 nm/380 nm at zero $[Ca^{2+}]$.

2. Cell viability assay. Culture plates of cells in the end point of treatment were removed of medium and washed with PBS for three times. Cells then incubated in PBS containing 1 μM Calcein AM and stay at room temperature for 30 to 40 minutes and avoid of light. Finally, plates were measured with fluorescence reader in 494/517 nm (Ex/Em), and the intensity of fluotescence was represent of cell viability..

V. 結果與討論：

1. Oxidized LDL induced cell death

There is an increasing body of evidence showing that oxidized LDL plays an important role in cardiovascular disease. It also has been shown that oxidized LDL could induce cell apoptosis in different cells via vary mechanism of action. In our study, we have successfully induced cell death in neonatal rat cardiaomyocytes (Fig 1.), and we also have proved that the process of oxidized LDL induced cell death of neonatal rat cardiaomyocytes is via programmed cell death – cell apoptosis.

2. The effect of oxidized LDL and glucose on cell viability

Besides the cytotoxicity effect of oxidized LDL, we also found that high extracellular glucose concentration may increase the cytotoxicity effect of oxidized LDL (Fig. 2). The actual mechanism is still unknown. However, it is also worth to notice about that the exposure to high concentration of glucose alone is sufficient to induce cell death. Some investigators have referred the phenomenon on other type of cells to the activation of p38 MAPK. We believe that there will be a cross talk between the signal transduction pathways of oxidized LDL and high glucose stimulation, and our finding may discover some complicate mechanisms of cardiovascular disease that associated with diabetes.

3. Measurement of Ca^{2+} transient of cultured neonatal rat cardiomyocytes

In muscle cells, the regulation of cell contraction was mediated by the intracellular Ca^{2+} concentration. In order to detect the change of Ca^{2+} transient, we use confocal microscopic system to scan the fluorescence emission of Ca^{2+} sensitive dye. The signal we recorded could provide as a functional assay of cardiac myocytes.

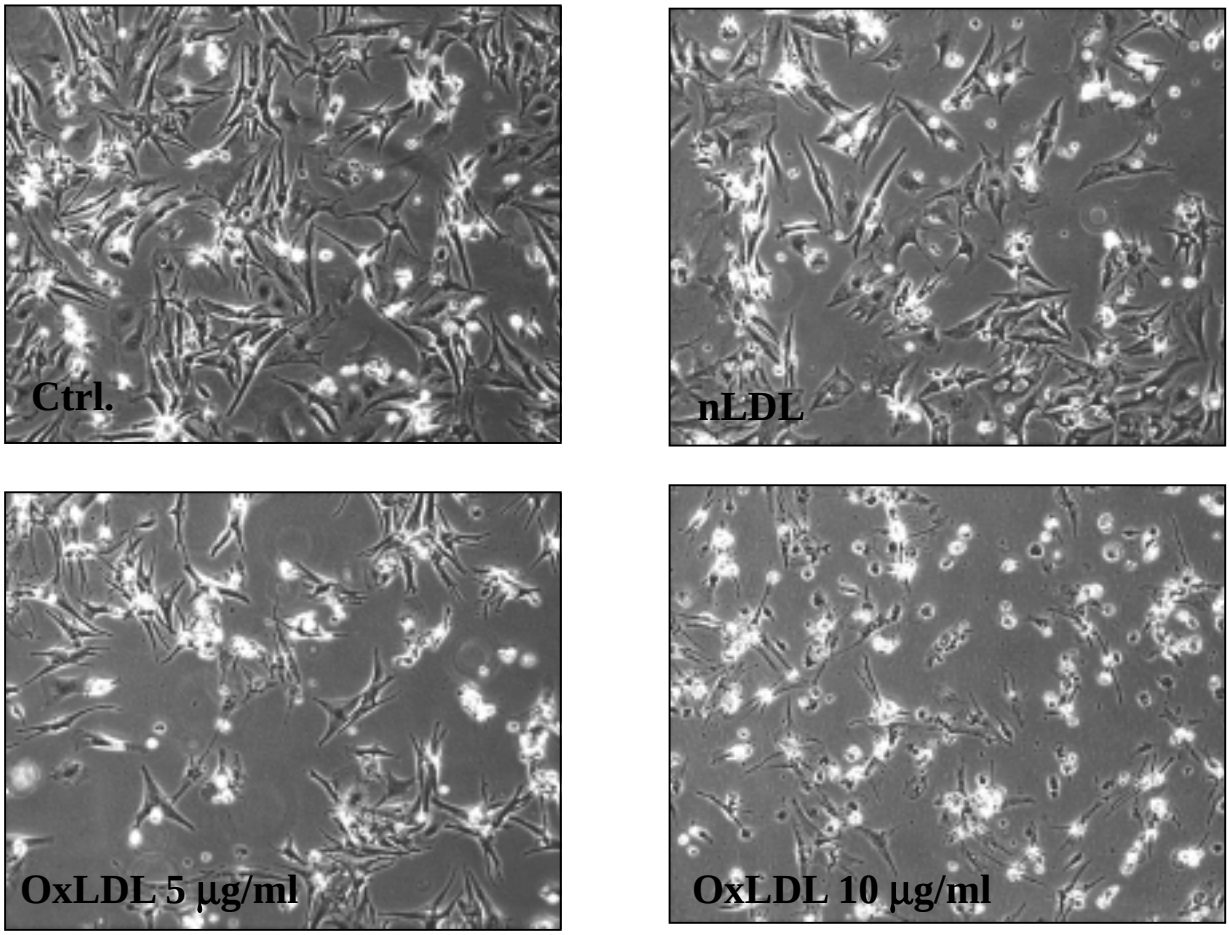

Fig. 1 Oxidized LDL induce cell death of neonatal rat cardiomyocytes. Figures represent the cytotoxicity effect of oxidized LDL in 24 hr treatment.

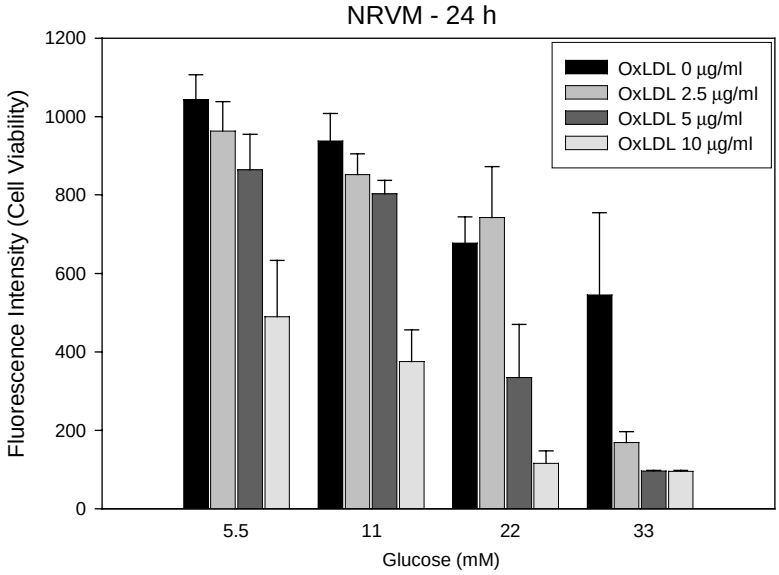

Fig. 2 The effect of oxidized LDL and glucose on cell viability. Increasing glucose concentration may also increase the cytotoxicity effect of oxidized LDL.

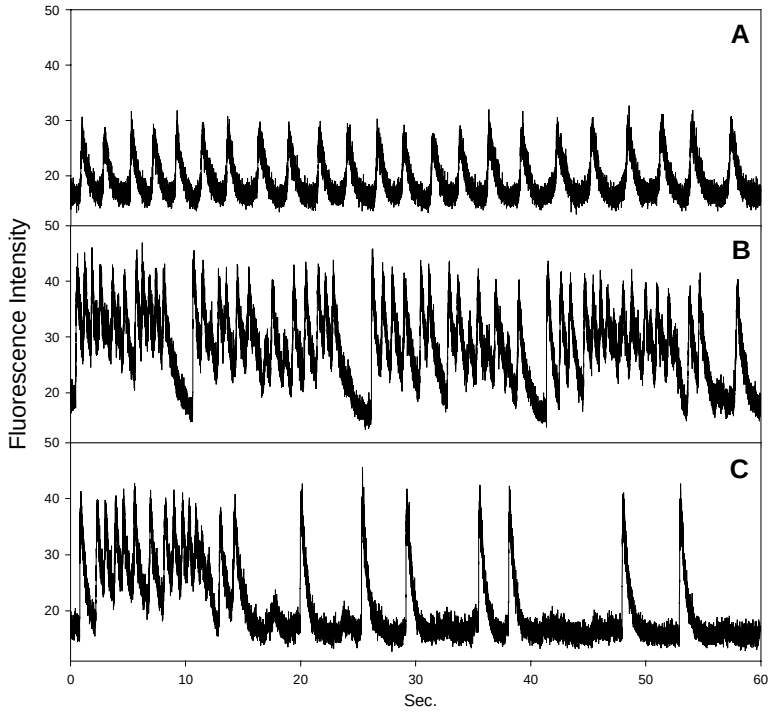

Fig. 3 PE induced intracellular Ca^{2+} concentration transients $[Ca^{2+}]_i$ in neonatal rat ventricular myocyte (NRVM) culture. **A**, $[Ca^{2+}]$i was recorded from a single fluo-3 AM-loaded NRVM. **B**, NRVM in 200 μM PE. **C**, NRVM in 300 μM PE.

四、參考文獻：

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五、計畫成果自評：

In these two years, we have set up the cell culture systems and investigated the interactions between endothelial cells and cardiomyocytes. Surprisingly, we found that different types of cells may have different response under same kind of stress. Our finding may provide impact on the clinical treatment.