行政院國家科學委員會專題研究計畫　成果報告

過氧化體增殖劑活化受器促進劑Rosiglitzaone對於大白鼠過度左心室後負荷所致心衰竭的心肌整體基因表現型態之影響

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-224-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學醫學院內科

計畫主持人：黃瑞仁

計畫參與人員：黃瑞仁

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 10 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

（計畫名稱）

計劃名稱

中文：過氧化體增殖劑活化受器促進劑 Rosiglitzaone 對於大白鼠過度左心室後負荷所致心衰竭的心肌整體基因表現型態之影響

英文：Global gene expression profiling of congestive heart failure subsequent to experimental left ventricular pressure overload pretreated with the peroxisome proliferator-activated receptor-gama agonist Rosiglitazone

計畫類別：☑ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 93－2314－B－002－224
執行期間：　2004 年 08 月 01 日至 2005 年 07 月 31 日

計畫主持人：黃瑞仁 副教授
共同主持人：
計畫參與人員：

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
處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：國立台灣大學醫學院內科
中　華　民　國　94　年　10　月　31　日

（一）摘要

中文摘要

關鍵詞：過氧化體增殖劑活化受器促進劑，過度壓力負荷，心臟衰竭，cDNA 微陣列技術，整體基因表現型態

高血壓一直為公衛上一個相當重要的議題。？所周知，高血壓是許多神經系統以及心血管系統的併發症的一個重要的危險因子。好好控制高血壓可以有效增進患者的預後。然而根據研究指出，仍然有三分之一的患者，其掏血壓仍無法被控制在滿意的範圍之內。長期未良好控制的高血壓將會使左心室的心室後負荷上升，心肌細胞為了維持心輸出量，心肌細胞會變的肥厚來應付所需的功，於是乎左心室肥厚便產生。而同時，纖維母細胞也會被活化，使的 collagen 產量增加，而使的間質增生亦隨之增加，並開始產生心肌細胞之纖維化。早期只是單純的心室肥厚，因此雖有心臟舒張功能失調，但是心肌之收縮功能正常；而到晚期，心臟的收縮功能亦會受到影響，而進展到代償不良之心臟衰竭。心肌梗塞後，細胞骨架和收縮元件的變化，與 Actin 和 myosin 基因表現改變相關，而 Collagen 和 fibronectin 基因表現則是梗塞組織纖維化的部份原因。梗塞後，為了改善血行動力，心肌組織中 atrial natriuretic peptide mRNA 及其蛋白質產物也會增加；而內皮一氧化氮生成？(eNOS)，細胞外間質(ECM) metalloproteinases (MMPs)和腫瘤壞死因子(TNF)的基因表現亦牽涉其中，同時存活的心肌組織也會變的更為肥厚。這些變化皆與心肌梗塞後心臟進行再塑型(remodeling)作用息息相關，也對梗塞造成之心臟衰竭演進有相當之影響。

過氧化體增殖劑活化受器(PPARs)屬細胞核內受器群，共分三種亞型α、β/δ、和γ，其為轉譯因子，可調節基因表現並控制不同方面的細胞功能，包含脂肪和脂蛋白的代謝，脂肪酸氧化，葡萄糖代謝，脂肪新生，細胞分化等等。近來研究亦發現 PPARs 表現也會影響免疫細胞、巨嗜細胞、內皮細胞和血管平滑肌細胞，並影響不同細胞之細胞激素(cytokines)分泌或其他酵素分泌(如 MMPs)，因此對血管硬化之調控亦有重要角色。PPARγ促進劑在動物模式顯示可減少心肌梗塞區域及收縮功能失常，且對於梗塞後左心室再塑型作用及心臟衰竭也具有減緩之作用。不僅如此，對於心肌肥厚的進展，PPARγ也在不同研究中顯示有減緩之作用。唯目前僅知道與部分細胞產物有關，對於長期左心室壓力過度負荷後心肌整體影響以及保護機轉明瞭則相當少。

cDNA 微陣列晶片(cDNA microarray)**可以同時分析成千的基因表現，對疾病致病機轉研究助益甚大。本研究希望藉此有效的研究平台來深入解析 PPARγ促進劑** Rosiglitazone **對心肌梗塞可能具保護作用的機轉以及其影響梗塞後心衰竭演進的機制，並希望應用到日後心肌梗塞之治療。**

英文摘要

關鍵詞 (Keywords): peroxisome proliferator-activated receptor-γ agonist, pressure overload,

congestive heart failure, remodeling, cDNA microarray, gene expression profiling

Aim of study: To explore the gene expression profiles of congestive heart failure (CHF) subsequent to experimental pressure overload pretreated with peroxisome proliferator-activated receptor-γ (PPARγ) agonist Rosiglitazone. And to elucidate the potential benefits and mechanisms of PPARγ agonist on pressure-overload hemodynamics, cardiac remodeling, and CHF.

Background: Hypertension (HTN) continues to be of major public health concern. Increased risks of several adverse neurological and cardiovascular outcomes were documented in the population of HTN. Longstanding HTN increased the left ventricular (LV) afterload markedly, and cardiac fibrosis evolves as an adaptive response to hemodyanamic overload. Subsequently, interstitial fibrosis occurred and contributed to LV stiffening and impaired compliance. Although initially the LV systolic function is well preserved with simply diastolic dysfunction, however progression to decompenated stage congestive heart failure (CHF) with systolic dysfunction is hastened. PPARs are nuclear receptors, which function as transcription factors the regulate different genes expression. They control a variety of cellular functions and are involved in control of vascular inflammation, thrombogenecity and inflammatory cytokines. Several animal studies had shown that ligands of the PPARs can reduce infarct size and potentially protect the heart against ischemia-reperfusion injury. PPARγ ligands have also been shown to suppress the development of cardiac myocyte hypertrophy both in vitro and in the in vivo setting. However, knowledge about the influences of PPARs on pressure-loaded CHF and cardiac remodeling process at cellular level is quite limited. cDNA microarray offers a genome-wide simultaneous analysis of gene expression profiling changes in perturbed physiological or pathological conditions. With the aid of this powerful tool, researches could progress efficiently.

Experimental protocol: Rosiglitazone-pretreated or placebo-preteated rats undergo abdominal aorta ligation resulting in left ventricular overload. Two-color cDNA microarray system containing 7,600 clones from regional company is applied to explore the cardiac differential gene expressions between Rosiglitzaone-pretreated and placebo-pretreated rat pressure overload model. Serum biochemistry, hemodynamics, echocardiography, and clinical conditions are also measured prior to sacrifice and will be analyzed.

Expected results: The two-color cDNA microarray data analysis will be validated utilizing RT-PCR. Through this study, Rosigliazone pretreatment effects, whether beneficial or not, on pressure overload cardiac remodeling and CHF course could be evaluated, and differential cardiac gene expressions contributing to the probable beneficial effect of Rosiglitazone on

CHF subsequent to pressure overload could be elucidated.

研究目的：

To explore the gene expression profiles of congestive heart failure (CHF) subsequent to experimental pressure overload pretreated with peroxisome proliferator-activated receptor-γ (PPARγ) agonist Rosiglitazone. And to elucidate the potential benefits and mechanisms of PPARγ agonist on pressure overload hemodynamics, cardiac remodeling, and CHF.

結果:

Table 1. Echocardiographic parameters

	Echo	Sham	Sham+Rosi	Banded	Banded+Rosi
0 week	IVS, mm	0.75±0.03	0.74±0.04	0.75±0.04	0.73±0.03
	PW, mm	0.75±0.02	0.75±0.07	0.75±0.03	0.73±0.03
	%FS	43.4±2.0	43.5±1.6	43.5±1.6	43.5±1.6
3 week	IVS, mm	0.74±0.03	0.75±0.03	0.96±0.03*	0.81±0.03**
	PW, mm	0.75±0.02	0.74±0.03	0.98±0.03*	0.84±0.01**
	%FS	43.1±2.1	43.3±1.9	39.2±2.0	41.2±1.8
8 week	IVS, mm	0.75±0.02	0.75±0.04	1.12±0.02*	0.95±0.04**
	PW, mm	0.74±0.03	0.75±0.03	1.14±0.03*	0.96±0.02**
	%FS	43.5±1.8	43.2±1.7	35.7±1.7*	38.2±1.8

Data are mean±SEM (n=6 in each group)

Table 2. Blood pressure recordings

	BP (mmHg)	Sham	Sham+Rosi	Banded	Banded+Rosi
0 week	SBP	115±5	112±4	112±4.4	114±3.9
	DBP	81±8.4	83±6.1	85±4.8	85±8.4
	MBP	95±6	94±7.2	95±5.2	96±7
3 week	SBP	110±5	110±6	157±11	160±10
	DBP	84±8.4	84±5.4	111±5	115±3
	MBP	94±6	94±4	131±7	135±2
8 week	SBP	118±5	116±4	151±10.2	147±8.8
	DBP	87±8.4	87±4.4	107±5.6	111±3.7
	MBP	98±6	97±8	123±6.8	129±4.2

Data are mean±SEM (n=6 in each group)

Table 3. Top significantly different genes between banded group and Rosi-treated banded rats.

Gene	Accession No.	Fold Change	P-value
Atrial natriuretic peptide (ANP)	M30262	3.1	2.8E-07
Interleukin-1 receptor-associated kinase	L76191	3.1	3.6E-07
Lectin P35	D63158	6.4	5.1E-07
Brain natriuretic peptide (BNP)	D16497	2.6	8.9E-07
Plasma glutathione peroxidase	U13705	2.2	1.2E-06
Fibroblast inducible specific protein 12	M70642	4.1	4.5E-06
Cardiac beta myosin heavy chain	AA73283	2.6	3.3E-05

90kDa heat-shock protein	X15183	2.2	4.1E-05
Elongation factor 2	X51466	24.1	0.0001
Ribosomal protein L39 homolog	L05096	9.1	0.0001
Skeletal muscle LIM protein 1	W14830	3.8	0.0001
Heparin-binding EGF like growth factor	U39192	3.1	0.0001
Actin 1	W55087	3.1	0.0003
snRNA activating protein complex 19kDa subunit	AF93593	4.1	0.0003
Muscle plasminogen activator inhibitor	M33960	2.8	0.0003
Histone H2a(A)-613, H2b(B)-613, H2b-613	U62673	2.1	0.0003
Endothelial actin-binding protein	W97775	2.0	0.0003
Vascular smooth muscle alpha-actin	X13297	2.3	0.0004
Peptidoglycine alpha-amidating monooxygenase	U79523	2.1	0.0004
Alpha-tubulin isotype M-alpha 2	M13345	1.7	0.0004
Follistatin-related protein	D89937		0.0004
Decortin	NM001920	9.7	0.0004
Calnexin	M94859	3.8	0.0005
Cyclophilin C	S71016	3.1	0.0005
Ubiquitin conjugating enzyme	AF032456	4.1	0.0005
Dihydrofolate reductase	J00140	2.6	0.0005
Thyroid associated ophthalmopathy autiantigen	S67069	2.2	0.0005
Elastin	M24782	24.1	0.0006
Fatty acid binding protein homologue	M94856	9.1	0.0006
Phosphoglucomutase 1	M83088	24.1	0.0006
Thymidylate kinase	L16991	9.1	0.0006
Antigen CD36	M98398	2.4	0.0006
Collagen alpha-1 type 1	M55998	1.7	0.0008
T-cell specific protein	M21121	3.5	0.0008
Cystatin B	L03558	4.1	0.0011
Eukaryotic initiation facto 4	D485387	2.8	0.0011
U4/U6 small nuclear ribonucleoprotein	AF16369	2.1	0.0011
Calcineurin inhibitor	W30069	2.0	0.0012
Mena protein	ET62477	2.3	0.0012

Dynein heavy chain, cytoplasmic	D13896	2.4	0.0014

計畫成果自評：

根據本動物實驗模式所找出來的基因群組，可以在人類高血壓族群之中進一步尋找血清標記，確定是否有可能成為檢驗或診斷工具的新工具。另外 Rosiglitazone **對左心室後負荷增加的情況(模擬人類高血壓族群)，數據顯示對大白鼠的血行動力學以及心室功能的確有改善現象。但是改善有顯著差異的部分主要在左心室功能，以及較改善的左心室嚴重肥厚現象。血壓的變化則與** banding **組無顯著差異。**Rosiglitazone **在本研究中顯示對左心室後負荷增加造成的左心室肥厚以及心室功能有正面助益，但是仍需要更多的實驗數據佐證方可更為確定之。而在** Microarray **實驗所找出的顯著差異表達基因，亦需要再進一步分析與致病機轉以及心臟衰竭進程之中的交互作用。**