中國醫藥大學針灸研究所碩士論文

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論文題目

穴位埋線與電針太衝穴治療保護自發性高血壓大鼠心臟效果及其分子機轉探討

Investigation the Cardio-protective Effects and molecular mechanisms of Catgut Embedding and Electro Acupuncture Therapy in TaiChong point of Spontaneous Hypertensive Rats

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致謝

誰說老狗難學新把戲? 雖然我年過半百,但在師長的教導及自己努力的學習下,我漸漸地克服對分子生物學及實驗室的恐懼。我真的愛上了我們的學校,不但圖書館的學習資源非常豐富， 研究所的師資也有相當高的水準。回顧這幾年來的學習課程,我的內心充滿了感恩。首先我要感謝天父的看顧， 雖然在我學習當中得了鼻咽癌， 但也讓我從中學習到珍貴的經驗， 這一切經驗與歷練將來必能成為他人的幫助。 感謝小兒科張正成醫師常到病房來帶領我禱告以及水滴浸信會主內弟兄姐妹們的代禱,主的恩點 使我的心靈從幽暗的蔭谷中得勝 ;亦感謝中國醫藥大學附設醫院我的主治醫師群蔡銘修主任、楊世能醫師 、曾憲彰醫師、廖裕民醫師和羅偉忠醫師等,因着他們的治療,我方能順利完成了 Tomotherapy 及化療， 直到痊癒， 因此我才能繼續做研究。 除此之外 我也要感謝蔡長海董事長、林正介院長、蘇家嫻護理長等在我住院期間的關懷與協助， 也感謝全校師生們的關心,我在此獻上最深摯的感恩,感謝您們讓我有機會健康快樂地活著再創生命的佳績。

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人，首推指導老師黃志揚教授及陳悅生教授，當我覺得信心不足時，老師總是給我安慰及鼓勵，若非老師協助與教導，指引論文方向 ，在學位取得上，必定無法順利。 感謝白培英醫師在百忙中幫我們測量老鼠的心臟超音波。我也要感謝分子生物學劉心屏教授、研究室助理楊家豪先生及博士班學姊沈美伶小姐等，因他們的耐心教導與協助， 我才能順利完成實驗分析 。我也要感謝婆婆及丈夫的支持鼓勵及照顧和所有曾經關心過我的親朋好友們， 謝謝他們一路上精神的支持，讓我感覺雖然辛苦但無負擔。 並以此論文獻給年邁的父親及在天上的母親!

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ABSTRACTS

(PART 1)

The Discussion of Electro-Acupuncture Treatment for Myocardial Hypertrophy and Cellular Apoptosis Caused by Hypertension..

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BACKGROUND: Hypertension is a condition defined by elevating blood pressure. About 90–95% of cases are termed "spontaneous hypertension", which refers to high blood pressure without any apparent medical cause. It can cause serious heart, brain and kidney dysfunction. Persistent hypertension may cause cardiac fibrosis, left ventricular hypertrophy and cardiac apoptosis, leading to the shortening of life expectancy. Clinical experiences have shown that the puncturing of Taichong point (LR-3) lowers blood pressure effectively.

OBJECTIVE: We punctured LR-3 point to observe the effect of lowering blood pressure and to explore the molecular mechanism of acupuncture

treatment for hypertension.

Acupuncture treatment for hypertension may have fewer side effects. This is the common point be used in clinic. According to the research, even using the single point of Taicong(LR-3) also can lower hyper-yang activity type hypertension well. We use SHR to make sure whether the effect of electro -acupuncture on the cardiac function of SHR has a positive impact. METHODS: A total of 12 rats with 8-weeks male spontaneously hypertensive rats (SHR) were randomized divided into the acupuncture and sham groups, with 6 animals in each group. Additional 6 male Wistar-Kyoto rats were served as the control group. Rats in the acupuncture group received electro-acupuncture at bilateral Taichong (LR 3) points. Their heart tissues were stained with Masson trichrome to observe whether there is myocardial fibrosis, and to observe myocardial cell apoptosis by TUNEL level. The expression of SOD1, FAS, FADD, Bax, cytochromC, Gαq, eNOS, and calcineurin and BNP were detected by Western blot.

RESULTS AND CONCLUSION: After treatment for three days, EAT group of blood pressure in rats that have decreased significantly, including systolic(BPs), diastolic(BPd) and mean blood pressure (BPm)were significantly lower than SHR and EAS group. At 19th day, the systolic blood

pressure in EAT group was still significantly lower than SHR and EAS group. Electroacupuncture LR-3 has shown that the blood pressure has gone down rapidly, but the long-term continuous stimulus, its antihypertensive effects would fade. Electroacupuncture LR-3 can reduce the damage of cardiac hypertrophy and apoptosis and fibrosis caused by hypertension. According to Western blot analysis, we found the eNOS and SOD 1 were increased. In another pathway,, the hypertrophy and apoptosis protein were decreased significantly.

Keywords: electro-acupuncture,LR-3, SHR, cardiac hypertrophy, apoptosis.

電針刺激降血壓及抗心肌肥大與細胞凋亡之探討

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背景：高血壓是因為全身動脈血壓升高的一種疾病。約 90-95 %的高壓病人找不到病因，是自發性的，所以被稱為“原發性高血壓“，它可以引起嚴重的心、腦、腎功能障礙。持久性高血壓有可能造成心肌纖維化、左心室肥大及心臟的細胞凋亡而使壽命縮短。在臨床上常有服西藥控制中的病人抱怨服藥所帶來的副作用。一個大量的臨床研究顯示，針灸太沖穴（LR-3）可以有效地降低血壓，可用於治療高血壓。

目的：我們通過電針刺激肝經的太沖穴來觀察降血壓的效果及對訊息傳導路徑的影響，而針灸治療高血壓具有療效顯著、副作用小、操作簡便等特點，並可以改善高血壓引起的症狀，是臨床上治療高血壓的常用方法之一 。根據研究獨取太沖穴治療肝陽上亢型高血壓 其降壓效果良好，降壓幅度與針刺前血壓呈正相關，不良反應少。我們用自發性高血壓大鼠（SHR）的動物模型來研究電針的效果是否對自發性高血壓大鼠的心臟功能有正面的影響。

方法：以 8 週大的雄性自發性高血壓大鼠隨機分為高血壓控制組(SHR)、電針偽穴位組 (EAS)、電針治療組 (EAT)，進行 3 週的治療。並以 8 周大的雄性 WKY (Wistar- Kyoto) 大鼠作為對照組。治療組大鼠在雙側太沖穴電針，並記錄各組大鼠的血壓變化。犧牲後取其心臟做切片，並觀察 H & E、Masson’s trichrome 染色與 TUNEL assay。游離出左心室並萃取蛋白質，進行西方墨點法，觀察 SOD 1、eNOS、肥大與凋亡訊息路徑蛋白質的變化。

結果與討論：治療第三天，EAT 組大鼠的血壓即有明顯下降，包括收縮壓、平均血壓與舒張壓都顯著低於 SHR 與 EAS 組。到治療第 19 天時 EAT 組大鼠只有收縮壓仍顯著低於 SHR 與 EAS 組。顯示電針太沖穴能達到迅速降血壓的效果，但長期的連續刺激，其降血壓效果會逐漸減弱。從切片可以觀察到電針太沖穴可降低高血壓對心臟造成的肥大、凋亡和纖維化的程度。西方墨點法分析發現經過治療的大鼠，eNOS 與 SOD 1 明顯增加，且肥大與凋亡路徑的蛋白質均明顯下降。

關鍵詞：電針，太沖穴，高血壓，自發性高血壓大鼠，心肌肥大，凋亡。

(PART Ⅱ)

The Comparison of Cardio-Protective Effects between Catgut Embedding and Electro-Acupuncture Therapies on the Taichong Point of Spontaneous Hypertensive Rats

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Advisor: Yueh-Sheng Chen

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Background: the persistent high blood pressure might cause myocardial fibrosis and left ventricular hypertrophy and apoptosis. Finally shorten the life. Objective: we learned from the previous experimental Taichong electro-acupuncture is effective for reducing blood pressure in spontaneously hypertensive rats, but long-term stimulation will make the effect fade in a row. So we designed a short experimental treatment and join discussions. Methods: the 8-week-old male group randomly divided into Spontaneous hypertensive rat group (SHR), pseudo-point electro-acupuncture Group (EAS), electro-acupuncture in the treatment group (EAT), pseudo-acupoint embedding Group (CES), embedding therapy group (CET), for 1 week of

treatment. With the 8-week-old male WKY (Wistar-Kyoto) as a control group of rats. Treatment group we chose bilateral Taichong point(LR-3) for electro-acupuncture and catgut embedding, and recorded their blood pressure with tail cuff. . After sacrifice we took its heart slices, and observed H&E, Masson's trichrome staining with TUNEL assay. Free out of the left ventricle and extraction of proteins, by Western blot to observe the proteins of anti-apoptosis and pro-apoptosis proteins and the proteins about hypertrophy or inflammation. Results and discussion: the treatment of the third day, the blood pressure of rats with has decreased significantly, including systolic, diastolic and mean blood pressure in EAT and CET group were significantly lower than SHR and EAS group, and the blood pressure in EAT group was slightly lower than the CET group , but no significant difference between the two groups. At the treatment of the 7th day EAT blood pressure in CET Group continued to decline, and in CET group blood pressure was less than EAT group but no significant difference between the two groups. We observed from the heart tissue biopsy proofed that the electro-acupuncture or catgut embedding on LR-3 can prevent cardiac hypertrophy and apoptosis and fibrosis from hypertension. Western blot analysis was found that the SOD 1 in CET group was significantly increased and apoptotic protein and hypertrophy and apoptosis protein were decreased

significantly, and SOD 1 and the induction of effect of PI3K/pPI3K in CET group were better than EAT group.

Keywords: electro-acupuncture, catgut embedding, Taichong point (LR-3), SHR, cardiac hypertrophy, apoptosis

比較穴位埋線與電針太衝穴對高血壓大鼠的心臟保護效果

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指導教授 ： 陳悅生教授

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背景：持久性高血壓有可能造成心肌纖維化、左心室肥大及心臟的細胞凋亡而使壽命縮短。目的：我們從先前的實驗得知電針太沖穴能有效降低自發性高血壓大鼠的血壓，但長期的連續刺激會讓此效果逐漸減弱。因此我們設計一個治療時間較短的實驗，並加入埋線治療的方式一起討論。方法：以 8 週大的雄性自發性高血壓大鼠隨機分為高血壓控制組(SHR)、電針偽穴位組 (EAS)、電針治療組 (EAT)，埋線偽穴位組(CES)、埋線治療組 (CET)，進行 1 週的治療。並以 8 周大的雄性 WKY (Wistar-Kyoto) 大鼠作為對照組。治療組大鼠在雙邊太沖穴電針/埋線，並記錄各組大鼠的血壓變化。犧牲後取其心臟做切片，並觀察 H&E、Masson’s trichrome 染色與 TUNEL assay。游離出左心室並萃取蛋白質，進行西方墨點法，觀察凋亡與抗凋亡訊息路徑蛋白質 and cardial hypertrophy and inflammation 結果與討論：治療第三天，EAT 與 CET 組大鼠的血壓即有明顯下降，包括收縮壓、平均血壓與舒張壓都顯著低於 SHR 與 EAS 組，且 EAT 組的血壓略低於 CET 組但兩組間無顯著差異。

到治療第 7 天時 EAT 與 CET 組大鼠血壓仍持續下降，且 CET 組血壓已低於 EAT 組但兩組間無顯著差異。從切片可以觀察到電針/埋線太沖穴可降低高血壓對心臟造成的肥大、凋亡和纖維化。西方墨點法分析可發現經過治療的大鼠， SOD 1 與抗凋亡路徑的蛋白質明顯增加，且肥大與凋亡路徑的蛋白質均明顯下降，且埋線對 SOD 1 與 PI3K/pPI3K 的誘導效果明顯優於電針。

關鍵詞：電針，埋線，太沖穴，高血壓，自發性高血壓大鼠，心肌肥大，凋亡。

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Part I.

The Discussion of Electro-Acupuncture Treatment for Myocardial Hypertrophy and Cellular Apoptosis Caused by Hypertension..

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Table of Abbreviation

ALS	amyotrophic lateral sclerosi
Bax	Bcl-2-associated X-protein
Bcl	B cell lymphoma
Bcl-2	B-cell lymphoma 2 gene
BNP	B-type natriuretic peptide
BP	blood pressure
Caspase	cysteinyl aspartate-specific proteases
CES	catgut embedding sham group
CET	catgut embedding treatment group
CKD	chronic kidney disease
CVD	cardiovascular disease
DAPI	4’, 6-Diamidino-2-phenylindole
DBD	DNA-binding domain
DBP	dystoloc blood pressure
DD	death domain
EAS	electro-acupuncture sham group
EAT	electro-acupuncture treatment group

ERK 5	Extracellular signal-regulated kinase 5
FADD	fas-associating protein with death domain
GPCR	G-protein-coupled receptor
H & E	hematoxylin and eosin
HTN	hypertension
IGF	insulin like growth factor
IGF-1	insulin-like growth factor 1
IGF-1R	insulin-like growth factor 1 receptor
IGF-2	insulin-like growth factor 2
IGF-2R	insulin-like growth factor 2 receptor
ISH	Isolated systolic Hypertension
IL-6	interleukin-6
JAK 2	Janus kinase 2
JNKS	the c-jun N-terminal kinases
LIF	leukemia inhibitory factor
LR-3	Liver Meridian 3, Tai Cong Point
LVH	left ventricular hypertrophy
MAPK	mitgogen-activated protein kinase
NOSs	nitric oxide synthases

eNOS	endothelial Nitric oxide synthase
iNOS	inducible Nitric oxide synthase
nNOS	neuron Nitric oxide synthase
OSA	obstructive sleep apnea
PI3K	The phosphoinositide 3-kinases
PKCα	protein kinase C α
PLCβ3	phospholipase Cβ
PVDF	polyvinylidene fluoride
SBP	systolic blood pressure
SHR	spontaneously hypertensive rat
SOD	superoxide dismutases
SOD 1	superoxide dismutase 1
STAT	the Signal Transducers and Activators of Transcription protein
TAD	transcription activator domain
tBid	truncated-Bid
TCM	traditional Chinese medicine
TNFα	tumor necrosis factor α
TNFR	tumor necrosis factor receptor

TRAIL	TNF-related apoptosis inducing ligand
TUNEL	Terminal deoxynucleotidyltransferasedUTP nick end labeling
dUTP	2’-Deoxyuridine, 5’-Triphosphate
WKY	Wistar-Kyoto

Material list

Materials	Manufacturer
2-Mercaptoethenol	J.T. Baker
3 mm chromatography paper	Whatman
Acrylamide-Bis 40 %	MDBio
Ammonium persulfate	J.T. Baker
Bromphenol blue	SIGMA
DAPI	Sigma
Diethyl pyrocarbonate	MDBio
Dulbecco's Phosphate-Buffered Saline	Invitrogen
Ethenol 95 %	TSC
Ethenol absolute	Panreac
Ethylenediaminetetraacetic acid	SIGMA
Formaldehyde Solution, 10% (w/v) in Aquenous Phosphate Buffer	Mallinckrodt Baker
Glycerol	Amresco
Glycine	MDBio
HCl	Showa
Hydrogen peroxide 30 %	SIGMA

Isoflurane	Abbott
Methenol	TSC
N,N,N',N'-Tetramethylethylenediamine	Alfa Aesar
Phosphotase inhibitor cocktail 2	SIGMA
Ponceau S solution	SIGMA
Protease inhibitor cocktail	Roche
Protein Assay dye reagent concentrate	Bio-Rad
PVDF	Millipore
skim milk	Anchor
Sodium dodecyl sulfate	Merck
Tris base	Usb
Tween-20	Showa
Xylene	Panreac

Equipment list

Instruments	Models	manufacturing companies
Luminescence analysis system	LAS-3000	FUJIFILM
Western blotting equipment		Bio-Rad
Homogenezator	POLY TRON	KINEMATICA
Anesthesia machine	Matrx vip3000	MIDMARK
Rodent ventilator	Model 683	Harvard Apparatus
Centrifuge	3700	KUBOTA
Fluorescence microscope		Olympus
Speedy autoclave	TM-328	Tomin medical equipment
Orbital shaker		Major Science
Heating block	Firefox Day Bath 6100	PANTECH
Hotplate stirrer	HTS-1003	HARMONT
ELISA reader	SPECTRAmax 340PC384	Moleculer Devices

Invasive blood pressure monitor	MK-2000	Muromachi
Electro-stimulator	Trio 300	ITO CO., LTD

Antibody list

1st Ab	MW (kDa)	2nd Ab	Brand	cat
β-actin (C-4)	43	Mouse	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-47778
Akt 1 (B-1)	60	Mouse	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-5298
pAkt (Thr 308)	60	Mouse	Cell Signaling Technology, Inc. Beverly, Massachusetts, USA	#9275
Bax (P-19)	23	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-525
Bcl-2 (C-2)	28	Mouse	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-7382
BNP (R-19)	36	Goat	Santa Cruz Biotechnology,	sc-18818

			Inc. Santa Cruz, California, USA	
Calcineurin	61	Mouse	BD Biosciences, San Jose, California, USA	610259
Cytochrome *c* (7H8)	15	Mouse	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-13560
FADD (S-18)	30	Goat	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-6035
FAS (FL-335)	48	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-956
Gαq/11 (C-19)	42	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-392
IGF-1Rα(H-78)	130/200	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-7952

IL-6 (H183)	21.5/28	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-7920
eNOS/NOS Type 3	140	Mouse	BD Biosciences, San Jose, California, USA	610296
pNOS3 (Ser1117)	140	Goat	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-12972
PI3K 85α (z-8)	80	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-423
pPI3K (Tyr508)	85	Goat	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-12929
pPKCα(Ser657)	82	Rabbit	upstate Biotechnology, Inc. Lake Placid, NY, USA	06-822
PLCβ3	150	Rabbit	Cell Signaling Technology, Inc. Beverly, Massachusetts, USA	#2482

SOD 1 (C-17)	23	Goat	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-8637
STAT 3 (K-15)	86/91	Rabbit	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-483
TNFα	26	Rabbit	Cell Signaling Technology, Inc. Beverly, Massachusetts, USA	#3707
α-Tubulin (B-7)	54	Mouse	Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-5286
Donkey anti-gout IgG antibody conjugated-HRP			Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-2314
Goat anti-mouse IgG antibody conjugated-HRP			Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA	sc-2005
Goat anti-rabbit IgG antibody			Santa Cruz Biotechnology,	sc2004

conjugated-HRP	Inc. Santa Cruz, California, USA	

Part Ⅰ.

Discussion of Electro-acupuncture Stimulation to Anti- myocardial Hypertrophy and Apoptosis due to Hypertensive Phenomenon.

電針刺激降血壓及抗心肌肥大與凋亡現象之探討

Chapter 1. Introduction

1-1 Definition of Hypertension

From western medicine of view, hypertension is blood pressure increased on the arterial wall, beyond the normal adjustment range, measure during different days twice or more times ,

Definition of hypertension in western medicine is that the systolic blood pressure is higher than 140 mm Hg, diastolic blood pressure is higher than 90 mm Hg, it can be diagnosed with high blood pressure. JNC6 (Table 1)[1] divided hypertension into two phases, namely Phase I (mild) hypertension (systolic blood pressure 140-159 mmHg and / or diastolic blood pressure 90-99 mmHg) and Phase II (moderate and severe) hypertension (systolic blood pressure equal to or higher than 160 mmHg and / or diastolic blood pressure equal to or higher than 100 mmHg). On the other side, the Traditional Chinese medicine (TCM) does not have the correspond to the name of disease .According to clinical symptoms, it manifested with headache or dizziness. What is the definition of hypertension in Traditional Chinese Medicine (TCM) point of view? According the classic book "Yellow Emperor" in the Ling Shu paragraph, Zhang Lun says that hypertension is “pulse expansion” (Mai Zhang) which means the pulse be felt in large,strong and unsmooth motivations[2] . This is the description of the pulse expansion, strong and slow blood flow conditions. Ling Shu says: the mind expansion (heart zhang), there will be upset, shortness of breath, disturbed during sleeping. This is due to excess pathogen like blood stasis or both qi and blood deficiency make the blood has poor circulation and then it will lead to the pulse pressure increase and pulse expansion appears.

Table 1-1. Categories for Blood Pressure Levels in Adults (in mmHg, or millimeters of mercury)

Category	Systolic (mmHg)		Diastolic (mmHg)
Normal	Less than 130	And	Less than 85
Pre-hypertension	130–139	Or	85–89
High blood pressure			
Stage 1	140–159	Or	90–99
Stage 2	160 or higher	Or	100 or higher

*When a patient's systolic and diastolic blood pressures fall into different categories, the higher category should apply. From JNC VI[1]

1-2.Type of Hypertension

There are three types of hypertension which are spontaneous hypertension (as same as essential hypertension or primary hypertension),secondary hypertension and isolated systolic hypertension(Fig 1-1). The etiology of spontaneous hypertension is still unclear so far. There are about 90–95% of cases are termed " essential hypertension", which refers to no medical cause has been found[3]. There is probably no any characteristic symptom and sign in the early stage of hypertension, but at the late stage, the peripheral vascular resistance will be increased. The clinical symptom in the middle stage of spontaneous hypertension is also extremely variable. The later stage could cause cardiac and vascular complication which includes disturbances of vision with severe headache, kidney blood vessels damage, cardiac function fails and cerebral haemorrhage.

Secondary hypertension is uncommon. The reported prevalence is only 5% to 10%. The most common reasons are chronic kidney disease (CKD), others such as renovascular disease, endocrine problems or obstructive sleep apnea (OSA) that affect the heart, kidneys, arteries, or endocrine system.[4]

The systolic blood pressure is means that the blood pressure higher than140mmHg with a normal diastolic blood pressure (<90).

General speaking, in TCM, hypertension can be categorized into many types according to his or her constitution. Those include normal constitution, qi deficiency, yang deficiency, yin deficiency, phlegm dampness, damp heat, blood stasis, qi stagnation and special constitution.

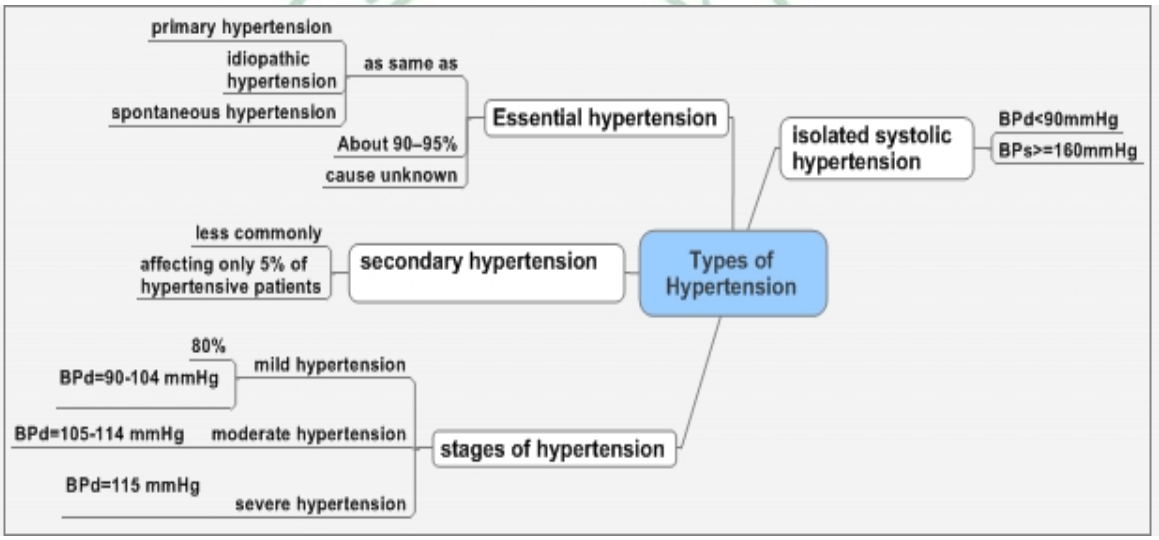


Fig 1-1. The type of hypertension

1-3 The Risk Factors of Hypertension

Risk factors for hypertension are not always direct cause of the disease, but seem to be associated in some reasons (Fig 1-2). Blood pressure tends to rise with age older than age 45 in male or than age 55 in female. It also related with race, it occurs more often in African American adults than in Caucasian. Overweight or obese is more likely to develop pre-hypertension. A number of lifestyle habits also can raise the risk for blood pressure such as stress,

taking high calories and heavy sodium or inactivity or always burn the midnight oil.

Although the primary hypertension remains unknown, the role of genetics, environment, and the gene-environment interaction is discussed. The key roles played by the central systems and peripheral nervous systems and circulating and tissue hormones are reviewed[5]

In TCM theory, hypertension could be caused by six evil qi , wind , summer heat, dampness, dryness, cold, fire pathogens invades body or caused by internal seven emotions, joy (a states of agitation or overexcitement) , anger, anxiety, pensiveness, grief, fear, fright. And also could be caused by neither internal nor external which is including dietary irregularities, overexertion and fatigue. Any one of above is possible make our body disharmony in yin and yang .Exuberance of yang leads to heat syndromes which marks hypertension with thirsty, irritability, red eyes, flush face, easy hungry, yellow or scanty urination or constipation etc. Exuberance of yin leads to cold syndromes which marks hypertension with cold limbs, aversion to cold, poor tasting, clear urination, lack of energy. Deficiency of yang leads to cold syndromes which marks hypertension with pale complexion, intolerance to cold, cold limbs, profuse and clear urination, loose stool with undigested food and so on. Deficiency of yin leads empty heat syndrome which makes hypertension with feverish sensation in the chest palms and soles, fever at evening time, emaciation, night sweats, dry mouth, flush on face, insomnia, seminal emission, dry mouth and throat, irritability and red tongue without any coating.

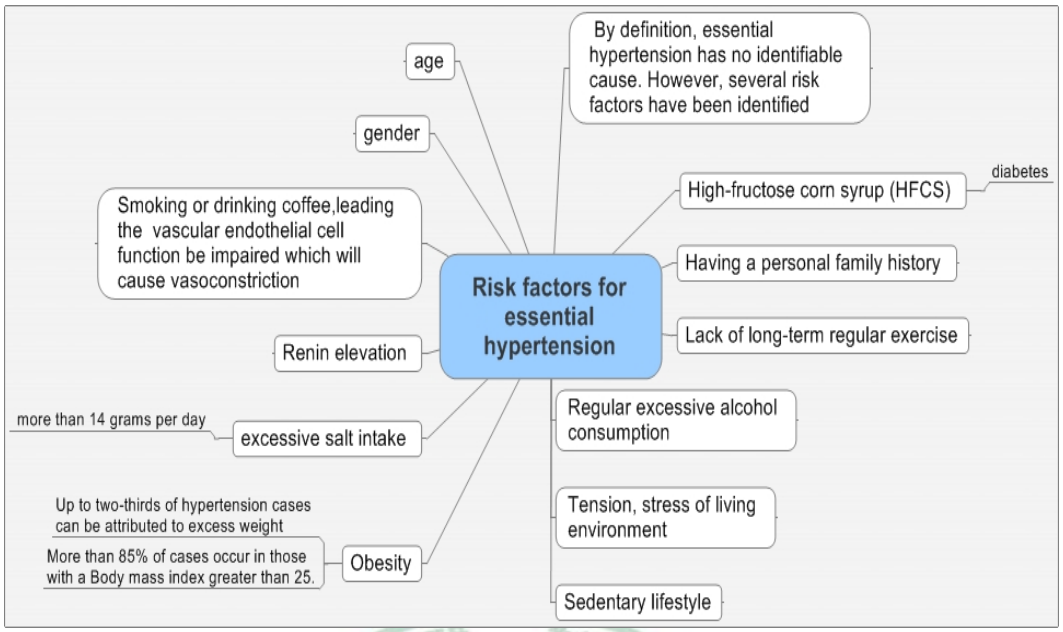


Fig 1-2.The risk factors of essential hypertension

1-4. The Symptoms of Hypertension

Hypertension is often called "the silent killer" because it may no symptoms at all, but organs and tissues might be damaged by hypertension without any manifestation.The symptoms of hypertension are difference from person to person (fig 1-3).

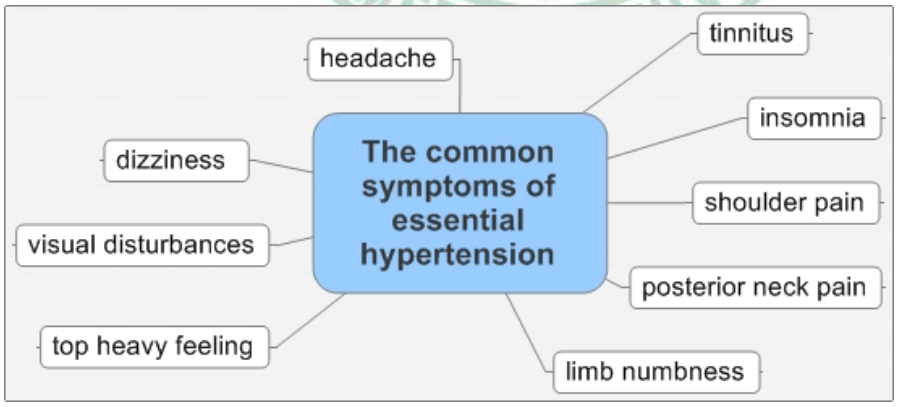


Fig 1-3. The common symptoms of essential hypertension

1-5. The Complications of Hypertension

Hypertension could be develop to the left ventricular hypertrophy (LVH), atherosclerotic heart disease, and even heart failure[6]. (Fig 1-4)

If arterial pressure in hypertensive patients were increased, it will cause whole body arteriolosclerosis and then affect the blood supply to tissues and organs, causing a serious of consequences, as the complications of hypertension. Various complications of hypertension, heart, brain and kidney damage is most seriously.

Left ventricular hypertrophy is major risk factor and it might caused sudden death by heart infarct and other cardiovascular events[6]

Hypertension can increase the risk of cerebral infarction or haemorrhage and also increase the risk of cerebrovascular morbidity and mortality and cognitive impairment and dementia [7]

In clinic, if hypertensive patient has dizziness, headache, nausea, numbness, fatigue and so on , the stroke may take place.

When the blood vessels in the kidney turn to weak and narrow, kidney complications will have occurred. Most forms of renal disease are associated with hypertension[8] When it happens, waste removal function from kidney becomes affected. Unfortunately, it has no any noticeable symptoms in the early stages of kidney problems caused by hypertension. On the other side, the retina's blood vessels be damaged, this may lead to headaches, blurred vision, and even blindness.

Acupuncture is one of methods to treat hypertension in traditional Chinese medicine(TCM). Ling Shu says that the twelve meridians can boost the blood circulation to nutrient the yin and yang, can moist tendons-bones to activate all joints. It also can determine the person's life and death, can treat most of diseases and can regulate excess and deficiency constitution, so, it can not be blocked. There are strong relationship between meridians and collaterals. So, when any organ has something wrong inside, it will be appear some symptoms. At the other side,

Acupuncture might able to prevent the heart turn to hypertrophy or heart failure or sudden death from long term hypertension. Catgut embedding (catgut implantation) is inject the catgut into acu-points to keep the point

stimulating until the catgut be absorbed[9]

Point combinations are commonly used in acupuncture to produce synergistic effects to enhance therapeutic benefits, but in this research, we just used one point of Tai chong (LR-3). It belongs to Shu-Stream, Yuan-Source Point . It locates between the 1st and the 2nd metatarsal bone on the dorsum of foot. The mainly of its function is smooth the liver qi and blood stagnation[10] .

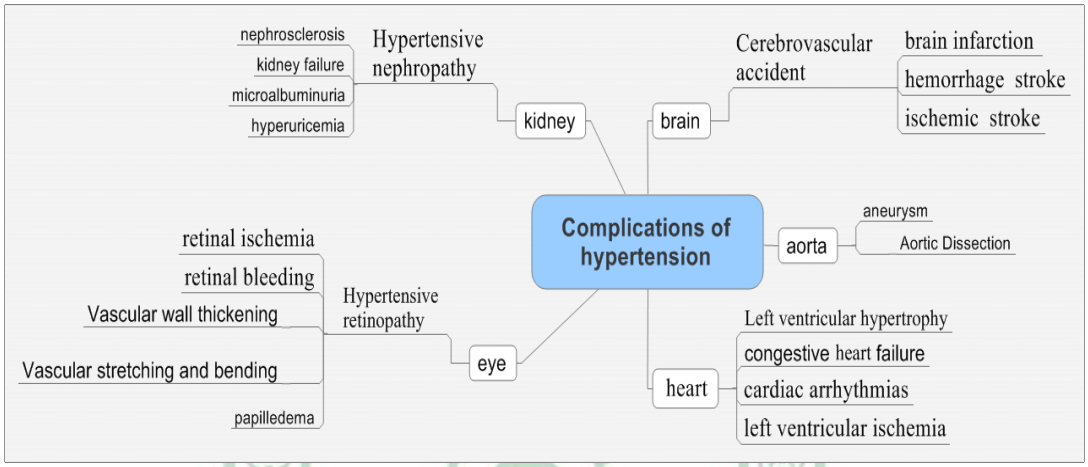


Fig 1-4. the complications of hypertension

1-6. Treatment Goals of Hypertension

The goal of antihypertensive cardiac function fails, therapy is reduce the cardiovascular and renal morbidity and mortality (Fig 1-5)[3]

During the initiation of prescription drug therapy, patients may get some side effect from the drugs or suffer from manifestations. In my point of view, acupuncture therapy could be a very good supplementary treatment. In addition to medical care, weight reduction and regular aerobic exercise, reducing sodium and sugar in the diet, discontinuing tobacco use and alcohol consumption, rich in fruits and vegetables and low-fat or fat-free dairy products, reducing stress, rich in potassium, magnesium, and calcium, as well as protein are recommended. In TCM conception, Hypertension is because of yin and yang imbalance plus long term mental stress or disturbed thinking, improper diet etc. which can cause liver fire flare up, liver yang

hyperactivity and kidney yin deficiency or phlegm damp obstruction. Acupuncture is used to prevent or treat diseases. It is possibly via regulating neural or humeral factors to improve microcirculation or Hemorheology and indirectly to the treatment of disease[11]. For this reason, acupuncture can be used in regulating blood pressure basically.

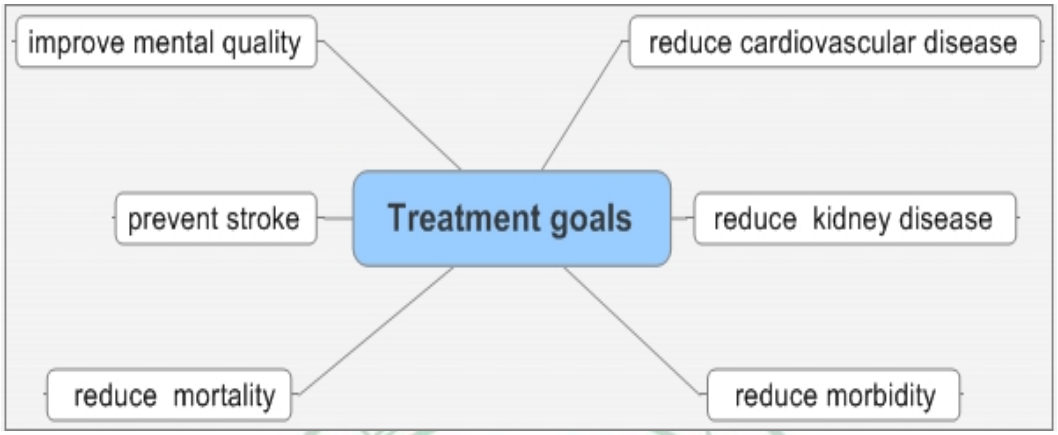


Fig 1-5. The treatment goals of hypertension

1-7. Nitric oxide synthases (NOSs)

NO released from endothelial cells mainly involved in regulating blood pressure and local blood flow regulation immediately

NO is the most physiologically active substances, and its main role is to cause blood vessels to dilate and the function of regulating blood pressure and platelet aggregation with the control function of the heart contraction. Numerous studies show that, NO in the cardiovascular, immune, nervous, digestive and other systems have an important regulatory role. Recent study found that NO can also regulate endothelial cell proliferation and metabolism and in vascular remodeling and regulation [12] .

There are 3 types of NOS be known in mammals: nNOS (neuronal NOS), iNOS (inducible NOS) and eNOS (endothelial NOS) [13]. iNOS and nNOS are secreted proteins, mostly present in the cytoplasm, nNOS are mainly located in the nervous system, relevant to their functions and communication between cells.

iNOS are mainly located in the immune system and cardiovascular system,

its main function associated with inflammation and immune responses. eNOS mainly locates in vascular endothelial cells, also has distribution in many organs, generating nitric oxide, with expansion of blood vessels function, also has the ability to influence many signal pathways.

In the cardiovascular, nitric oxide can regulate blood pressure and constant stability in maintaining the vascular tension. It plays an important role. Nitroglycerin in the treatment of angina pectoris since its in vivo into NO, and it makes the blood vessels dilated [14].

In the immune system, NO has the action with killing anti-bacteria, virus and tumor cells. In the nervous system, nitric oxide can promote learning and memory, and can regulate cerebral blood flow

1-8. Superoxide dismutases (SODs)

SOD has a special physiological activity, it is a metallic element that contains the activity in vivo protease. It is the primary substance which can clear out the free radicals. Research data show that [15, 16]: Under normal circumstances the body contain various organizations, including the serum activity of antioxidant enzymes must be timely and effective way to remove oxygen free radicals. The superoxide anion O2- can be gathered on the surface, heart, blood vessels, liver and brain cells. It was deposited in the vascular wall, will cause vascular fibrous, leading to artery hardening, hypertension and myocardial infarction.

Superoxide dismutase in the human body also contains three classes: SOD1 positioning in the cytoplasm; SOD2 is in the mitochondria; SOD3 is outside the cell. SOD1 is a Dimer, while the other two classes as a tetramer. Active site of SOD1 and SOD3 are contain copper and zinc, while SOD2 has manganese. SOD may counteract fibrosis, it is possibly by myofibroblast restored to fibroblasts.

The mice after birth if lack of SOD2 will die from severe oxidative stress; mice lacking SOD1 can cause many diseases, including liver cell

carcinoma[17],muscles accelerate erosion , early cataract occurrence [18]
In humans, SOD1 mutations may cause amyotrophic lateral sclerosis (ALS). Overexpression of SOD1 is common in patients with down's syndrome [19]. SOD also has anti-fibrosis effects, it's probabaly through myofibroblasts restored into fibroblast[20]

1-9. Cardiac hypertrophy

Normal left ventricular wall thickness, while the diastolic is generally no more than 13 mm. Characteristics of left ventricular hypertrophy can be divided into concentric, eccentric and physiologic[21](Fig 1-6). Concentric left ventricular hypertrophy is due to the left ventricular got a long term pressure overload, such as hypertension, leaving the compensatory thickening of the heart but the chamber size may be normal. Eccentric left ventricular hypertrophy due to the long-term left ventricular volume overloading while the size of the chamber changes, such as valvular regurgitation, however, the myocardial thickness may be normal. Physiological left ventricular hypertrophy is results in long-term training , such as athletes, the left ventricular wall thickening changes in both visible and heart chamber increases, but this change can be rapid recovered when stop training and ventricular wall thickness are not greater than 13mm[22]. Cardiac hypertrophy is an important adaptive response against outside stimuli like genetic heart defect, cardiac infarction, hypertension or diabetes[23].. Overloading of the heart would cause degeneration of cardiac remodeling, fibrosis, heart failure and even sudden death[24]. Cardiac hypertrophy activates signal transduction via outside stimuli and modulate protein synthesis, sarcomeric assembly, and gene expression[25]. In the experiment of culture of cardiomyocyte, many molecular had been found that could affect myocardial hypertrophy, e.g. catecholamines, cytokines, growth factors, hormones and vasoactive peptides, and simultaneously participate in signal transduction pathways of the cell.

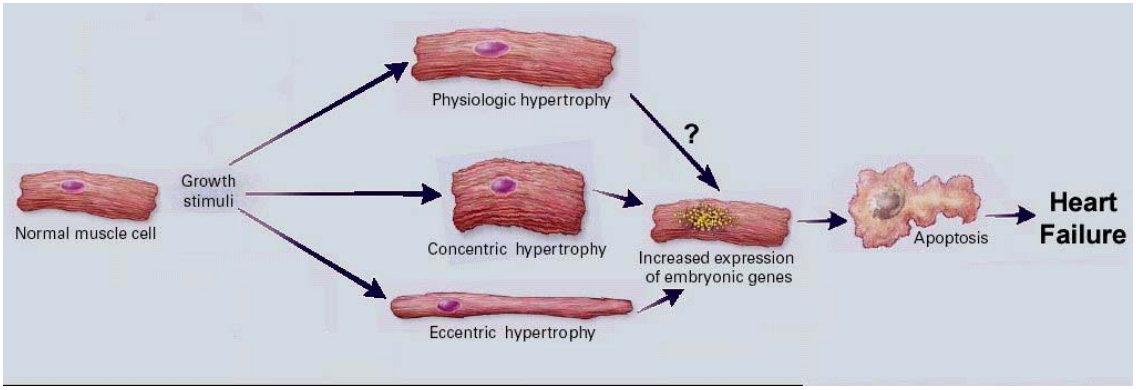


Fig 1-6.Cardiac hypertrophy progress[26]

1-9-1. Hypertrophy and Natriuretic peptides

Natriuretic peptides are a group of vasoactive hormone sharing similar sequence of peptides. For now, four types of Natriuretic peptides were found and named form A to D. These peptides could control function of cardiovascular, endocrine, and kidney by binding with receptors which could be classified into type A-C. Major role in the regulation of water balance and sodium and diastolic blood cell proliferation and differentiation[27]. Functions and locations of these peptides are shown in Table 1-2[28].

Table 1-2. Natriuretic peptide origin, stimulus for release and biologic effect

Natriuretic peptide	Location(s) of peptide	Stimulus	Effect
Atrial natriuretic peptide (ANP)	Cardiac atria	Increased atrial stretch and tension	Decreased plasma volume and blood pressure
B-type natriuretic peptide (BNP)	Cardiac ventricle	Increased ventricular wall tension	Decreased plasma volume and blood pressure
CNP	Heart, brain, kidney, vasculature	Shear stress	Vasodilatation, possibly acts as system neurotransmitter
D-type natriuretic peptide	Unknown	Unknown	Vasodilatation
GuanylinUroguanylin	Gastrointestinal mucosa	Unknown	Regulates salt and water transport
Adrenomedullin	Adrenal medulla, cardiac ventricles, lungs, and kidneys	Unknown	Reduction in plasma volume, blood pressure, vasodilatation

American Journal of Kidney Diseases[28]

1-9-2. IL-6 and hypertrophy

Recently, reports showed that cardiac hypertrophy would cause inflammation and release of cytokines like TNF-α (tumor necrosis factor alpha) and IL-6 (interleukin-6)[29] to induce a cascade of signal pathway. Some diseases can cause higher level of IL-6 in the blood such as atherosclerosis [4], metastatic cancer[30] , diabetes [31], systemic lupus erythematosus [32], rheumatoid arthritis. [33],Alzheimer's Disease[34] depression [35] and prostate cancer [36] .There are two hypertrophy pathways found to be associated with IL6, including IL6-MEK5-ERK5 and IL6-JAK2-STAT3.

ERK5 is one of Mitogen-activated protein kinases (MAPKs), MAPK pathway has three major cascades: 1. the extracellular-regulated kinases (ERKs) cascade; 2. the c-jun N-terminal kinases (JNKs) cascade; 3. the p38 cascade. Some test about transgene mice showed these 3 MAPK cascades would induce myocardial hypertrophy, but the mechanism about how these cascades affect hypertrophy is unclear. ERK5 belongs to MAPK1 (BMK1) and has a novel C-terminal and loop-12 domain. MEK5, the upstream of ERK5, is a highly specific MAPK kinase (MAPKK) which just phosphorylates ERK5 but not other MAPKs. Some growth factors stimulated by oxidative stress would induce activation of tyrosine kinase or G protein coupled receptor and cause the MEK5-ERK5 pathway[37]. Some reports showed that MEK5-ERK5 could promote myocardial sarcomere assembly[38], elongation of cardiomyocytes, and cause dialation of ventricular to eccentric hypertrophy (Fig 1-7)[39].

Janus kinases (JAKs) are protein tyrosine kinases binding to cytokine receptor (e.g. IL-6R and gp130) for regulating signal transduction. The MW of JAKs is about 120-130 kDa. Signal transducer and activator of transcription proteins (STAT proteins) in cytosol are in response to cytokines and growth factors. There are seven STAT family members which have been identified: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6[40].

The JAK/STAT pathway is mediated through receptor (gp130). Active JAK phosphorylates STAT protein which promotes the dimerization of STAT monomers via SH2 (Src homology) domain. Most references showed that STAT 1 participates in apoptosis[41], whereas STAT 3 plays a role in cardiac hypertrophy[42] (Fig 1-7).

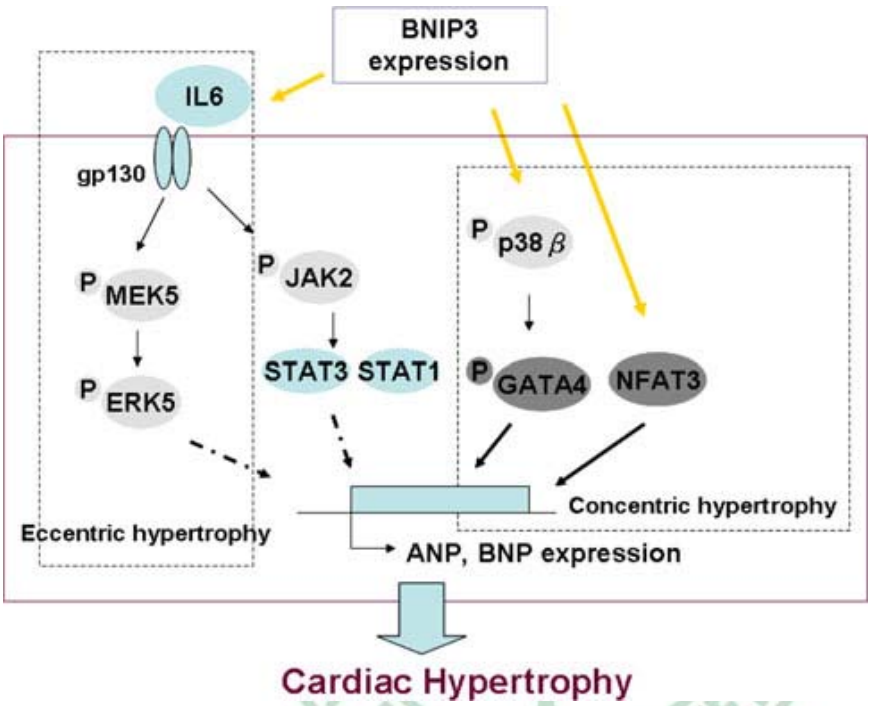


Fig. 1-7 cardiac hypertrophy pathway

BNIP3 protein belongs to pro-apoptotic proteins. BNIP3 in hypoxia can promote the expression of mRNA and protein induced apoptosis[43]

BNIP3 induced apoptosis is through mitochondrial gland pathway . BNIP3 in the cytoplasm combined with mitochondrial gland to damage mitochondrial function leading to mitochondrial gland cell death . During it is localized in the nucleus the apoptotic function is inhibited.

1-10. apoptosis

Signal of cell apoptosis was induced by a group of protease, caspases. Caspases (Cysteinyl aspartate-specific proteases) were specific cysteine proteases, specifically cutting aspartic acid residues. When cells were stimulated by external irritant, Caspases would be activated and induce apoptosis [44]. There is a cysteine at catalytic center of caspase, which cleave the aspartate of protein substrates. Caspases were classified to function, including initiator caspases (Caspase 2, 8, 9, 10) and effector caspases (Caspase 3, 6, 7). Initiator caspases working upstream of effector caspases Initiator caspase can transducer apoptotic signals from cell membrane and activate effector caspases, e.g. Caspase 8. Effector caspases manage the central mechanism of apoptosis, e.g. Caspase 3. When a caspase was activated, it would cause a cascade of downstream signals and result in apoptosis. There are 3 pathways to activate Caspases, including of Growth factor deprivation、Death receptor dependent pathway and Mitochondria dependent pathway[45, 46]

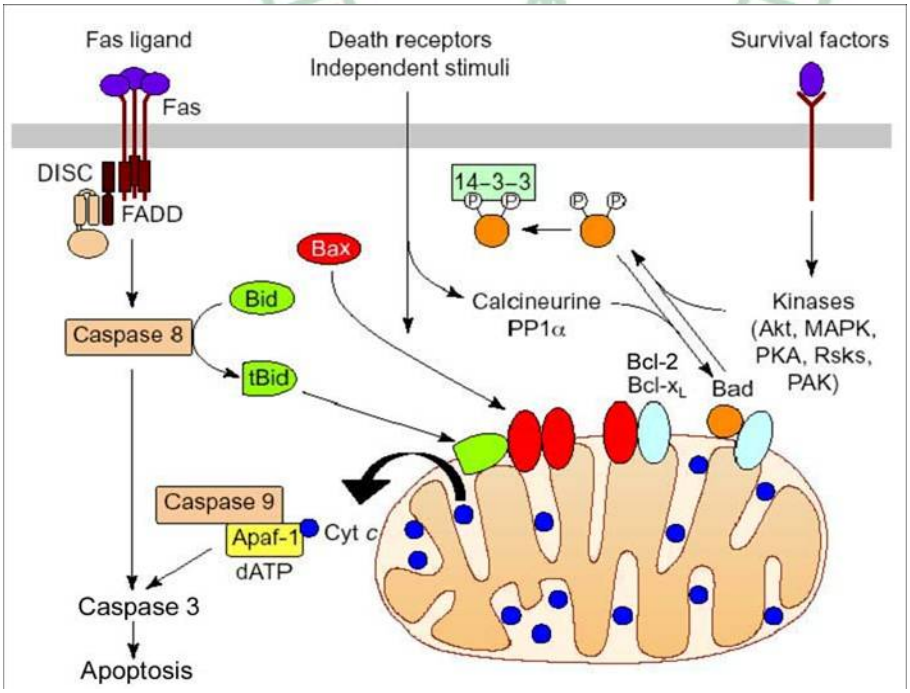


Fig 1-8. Death receptor dependent/independent apoptotic pathways[47].

1-10-1. Growth factor deprivation apoptotic pathway

Generally, growth factors can promote some kinases (e.g. MAPK; Mitogen-activated protein kinase) phosphorylating Bad to keep phospo-Bad in cytosol, not in mitochondia. If growth factors are blocked, Bad can bind with Bcl-xL on mitochondrial membrane and induce apoptosis [48]

1-10-2. Death receptor dependent apoptotic pathway

Death receptor contains a 80 amino acids intracytoplasmic death domain[49] This domain is rich in 4-6 Cysteine repeats, supporting the binding site with ligand to Death receptor. Death receptor is a transmembrane protein, and it's cytosol part contains a highly conserved Death domain (DD), supporting the binding site to other proteins with DD to induce downstream signal transduction.

Death receptor belongs to a larger group of TNF (Tumor necrosis factor) receptor, including Fas, TNFR1, DR3, DR4 and DR5. Therre are some homeotic ligands – Fas ligand (FasL, Apo-1), Apo-3L, Tumor necrosis factor-α (TNF-α) and TNF-related apoptosis inducing ligand (TRAIL)[50] For example, Fas is a prototypical death receptor. It can regulate cell survival by stimulated with FasL. Fas can form a trimer after binding with FasL, and this interaction results in the formation of the death-inducing signaling complex (DISC), which contains the FADD, pro-Caspase 8[51]. Active-Caspase 8 can cut off the N terminal of Bid to truncated Bid (tBid), which translocates to mitochondria membrane and induce the oligomeration of other pro-apoptotic proteins, resulting the release of Cytochrome c (Cyt c). Released Cyt c interacts with Apaf-1 (apoptotic protease-activating factor 1) and activates Caspase 9 and Caspase 3, resulting apoptosis[52] In another way, Caspase 8 can directly activate Caspase 3 and induce apoptosis (Fig 1-8).

1-10-3. Death receptor independent apoptotic pathway

This pathway is also called mitochondria dependent apoptotic pathway. In this pathway, Bcl-2 family proteins play a pivotal role[52]. According to functions, Bcl-2 family proteins are classified into two group: pro-apoptotic proteins (e.g. Bad, Bak, Bax, Bid, Bik) and anti-apototic proteins (e.g. Bcl-2, Bcl-xL, Mcl-1) . The members of Bcl-2 family share 1-4 conserved region, called Bcl-2 homology domain 1-4 (BH1-4) (Fig 1-9). Anti-apoptotic proteins, e.g. Bcl-2, Bcl-xL, contain a transmembrane domain at C terminal and locate on the mitochondria outside membrane by that. Anti-apoptotic proteins can keep the stability of mitochondria membrane voltage and inhibit the release of Cyt c[53]

All pro-apoptotic proteins contain a BH3 domain necessary for dimerization with other proteins of Bcl-2 family. When triggered by upstream signals, BH3 only pro-apoptotic proteins translocate to the mitochondria and initiate oligomeration of pro-apoptotic proteins, resulting in unstability of the membrane voltage and Cyt c releasing to the cytosol. Cyt c intergrates with Apaf-1 and pro-Caspase 9 to form Apoptosome, which combine pro-Caspase 3, and then Caspase 9 and Caspase 3 are activated[38]. Active-Caspase 3 turn on downstream signal cascade to induce cell death (Fig 1-8).

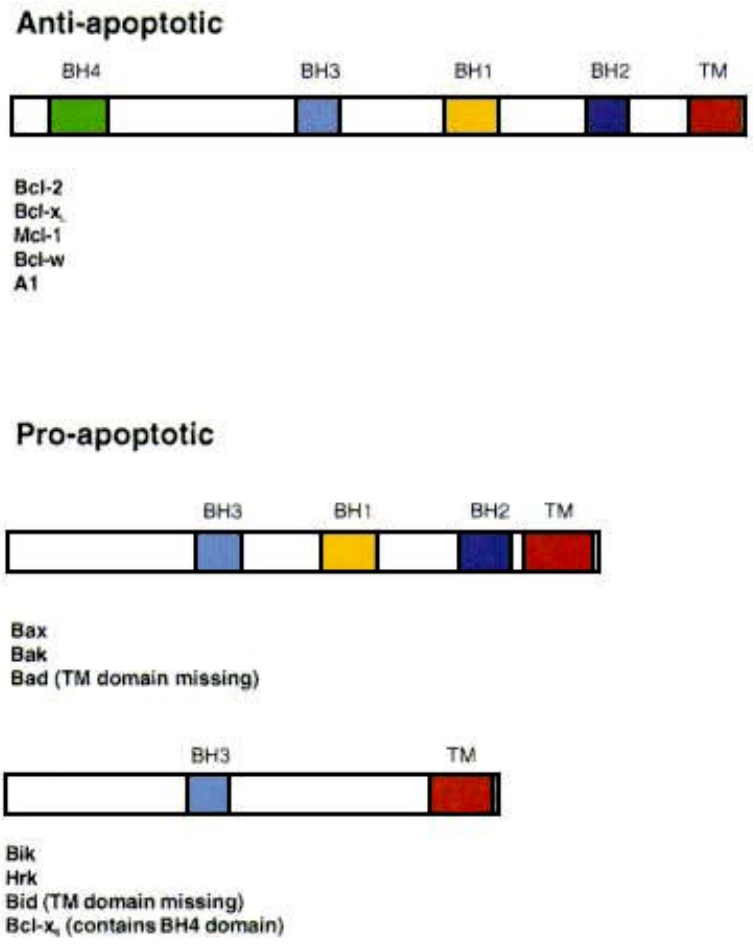


Fig 1-9. The structure characters and classification of Bcl-2 family

. BH, Bcl-2 homologous domain; TM, transmembrane domain[54].

1-11. insulin like growth factor, IGF

Insulin-like growth factor and insulin from the homologous genes. Which insulin is synthesized in pancreatic ß cells in proinsulin.[55] Growth factors can promote cell replication and proliferation, including insulin-like growth factor type I (insulin-like growth factor 1 IGF 1) in heart failure and ventricular re-engineering (ventricular remodeling) play an important role. In animal studies show IGF 1 can promote cardiac hypertrophy, increased cardiac contractility, inhibition of myocardial apoptosis and improve cardiac function. Clinically, patients who have heart failure will have low serum concentration of IGF 1, giving a small amount of IGF 1 in clinical trials and animal studies showed that can be improved in ventricular function. IGF-Ⅱ

is an embryonic gene.， During fetal growth and development IGF-II has an important function such as cardiac development, but it almost no expression after birth. However, when the case is in verge of death , it will over express in the heart. In the past, we speculated that the overexpression of IGF-Ⅱ may have similar IGF-Ⅰ in the aspect of anti-apoptotic activity, but previous studies in our laboratory confirmed that the combination of IGF-Ⅱ and IGF-2R in the heart can cause apoptosis. [IGF2R-1]. When the IGF-2R is activated , it interacts with Gαq , then Gαq activates PLCβ, which led to PKCα / CaMK Ⅱ phosphorylation and increased intracellular Ca2 + concentration. Ca2 + concentration increases and then Calcineurin will be activated. Active-Calcineurin would dephosphorylate pBad into Bad which translocates to mitochondria membrane and makes the membrane voltage unstable. Finally, cytochrome c is released and the mitochondria apoptotic pathway is activated[56] (Fig 1-10 A).

Previous research in our lab also indicated that IGF-2R activation would lead to cardiac hypertrophy followed with activation of PKCα/CaMK Ⅱ and up-regulation of ANP and BNP (Fig 1-10 B). [IGF2R-2]

A. B.

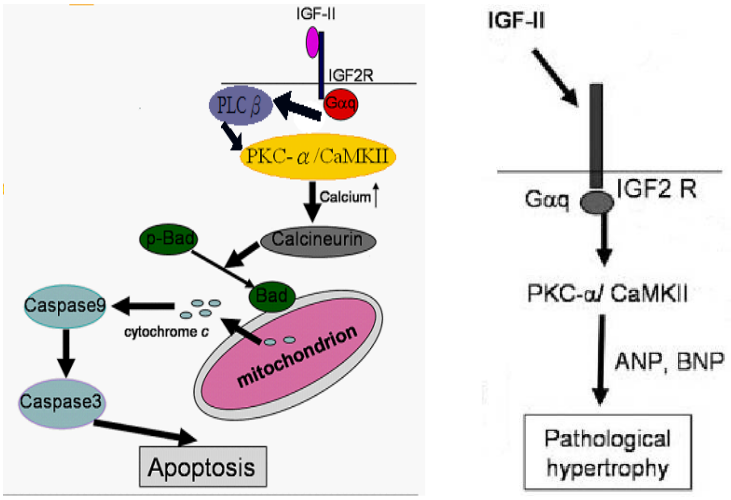


Fig 1-10. IGF-2R dependent cardiac signal pathway.

A) IGF-2R dependent cardiac apoptotic pathway[56].

B) IGF-2R dependent pathological hypertrophy pathway[57].

Chapter 2. Material and Methods

2-1 Source of experimental animals

8-week-old male SHR (Spontaneously hypertensive rats / Charles River Laboratories, SHR / NCrlNarl) rats and 8-week-old male WKY rats (Wistar-Kyoto, WKY / CrlBltw) were purchased from the National Science Council of the National Laboratory Animal Center. The rats housed at a controlled ambient temperature of 21-23℃ with 50 %-60 % relative humidity and a 12 hr/12 hr light (9:00-21:00)/ dark (21:00-9:00) cycle. The rats were fed with pelleted rat chow and RO water at libitum. After the adaptation for 1-2 weeks, we measured their tail pressure separately to make sure that blood pressure of the SHRs were significantly higher than WKY rats, and then went on the next steps to group them for the follow-up experiment.

2-2 Experimental animal groups

1. WKY: WKY male rats, as the negative control group with normal blood pressure.
2. SHR: SHR male rats with spontaneous hypertension as a positive control group.
3. EAS: SHR male rats, and gave acupuncture treatment on sham-points.
4. EAT: SHR male rats, and gave acupuncture treatment on Taichong Point.

2-3 the animal experiments

After adaption for 1-2 weeks, we randomized them into the four groups including the WKY group (WKY), the SHR untreated group (SHR), electro-acupuncture sham-point group (EAS), and electro-acupuncture treatment group (EAT). For treating the rats, they were anesthetized with isoflurane, and then we punctured them in the pseudo-points or Taichong point (LR-3) with electrical stimulation. The EA groups were treated once per day. We measured their tail pressure at the 0, 3rd and 19th day. After the

treatment for a week, all the rats were sacrificed, and their hearts were harvested. One of each group was put in 10 % formalin and the others were stored in -80 ℃

2-4 The treatment

2-4-1. Locations of treatment points

A. Taichong point: It locates on the dorsum of the foot, between the first and second metatarsal bones, in the depression anterior to the combining site of the base of metatarsal bones. Depth of about 2-4 mm

B. Sham-Point: We chose the point was on the foot dorsal, between the third and fourth metatarsal depression in front of the bottom junction, depth of about 2-4 mm.

2-4-2. EA parameters

The rats were quickly anesthetized with 5 % isoflurane mixed with oxygen, and then changed to 2-2.5 % to maintain anesthesia. Before puncture, we sterilized the local region with 75 % alcohol, inserted the needle of 32 G * 0.5-inch size respectively, and then connected to the positive and negative electrodes, and followed the parameter settings: 2 Hz / 1 mA/150 ms, 30 minutes has been set for every treatment per day.

2-5 stained tissue sections

After sacrificed, we washed out the blood with cold PBS and got their heart tissue, then soaked in 10 % (w/v) formaldehyde at least 24 hours. The heart samples were sent to Changhua Christian Hospital to have embedded, sliced, and analyzed with Hematoxylin and Eosin stain (H & E stain) and Masson's Trichrome stain. H & E stain and Trichrome stain of the biopsies were observed and recorded with 400 times of the microscope (Olympus).

2-6. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL assay)

Sections were incubated in the hybridization incubator at 58℃ for overnight. Next day, sections were put into 100% Xylene for 5 min (repeat thee times), and then put in 100 %, 95 %, 90 %, 85 % and 75 % alcohol each for 5 minutes sequentially, and finally washed into ddH2O repeatedly twice for 5 minutes. This process was for dewaxing and rehydration. Removed the slices and wipe the slices around with lens tissue around carefully (don't let the slices dry out). Use the DaKo pen to fence the scope of the sections. We covered the sections with proteinase K for 30 min in dark (range of coverage can be sliced off), then washed in PBS for 5 minutes twice. Added 3 % H2O2 for 10 minutes and washed with PBS for 5 minutes twice. Reacted with the permeabilisation solution (0.1 % sodium citrate) for 8 minutes and repeated washing step. Reacted with the blocking buffer for 1 hour, and washed with PBS for 5 minutes twice. Reacted with the mixture of the Enzyme Solution and Label solution (in 1:9 ratio) (In Situ Cell Death Detection Kit, 11684817910, Roche, Mannheim, Germany) in dark for 1 hour at 37 ℃, and then washed with PBS for 5 minutes twice. Reacted with DAPI stain (D9564, Sigma, USA) diluted to 20,000 times by PBS in dark for 5 min, and then washed with PBS for 5 minutes twice. Finally, the sections were observed and recorded with the fluorescent microscope.

2-7. process of disengaging for the left ventricle

After the animal models sacrificed, took their heart tissues and soaked in the icy PBS and then washed out blood, moved off the blood vessels which attached to the organs, also clipped the fat tissues and other connective tissues and then made them in dried condition. Cut away the left and right atrium and right ventricle. Finally, divided the disengaged left ventricle into equal portions, the weight for each part was about 0.1 g. All of them were put into centrifuge tubes and stored in -80℃.

2-8. extracting the protein of LV

Got 0.1 grams of left ventricular tissue , added 1 mL homogenization buffer (20 mM Tris, 2 mM EDTA, 10 % glycerol, 50 mM 2-mercaptoethanol, protease inhibitor, 1/1000 phosphatase inhibitor, pH7.4), put on the ice, ground by tissue homogenizer, Centrifuged and rolled for 12,000 rpm at 4 ℃ for 40 minutes and repeated above for wice, then stored in -80℃ for overnight, Repeated above once again and then got supernatant fluid. This is the tissue protein solution.

2-9. Bradford protein assay

The quantitative concentration of protein we used Bradford protein assay. The basic principle is according to Coomassie Brilliant Blue G-250 can easily bind protein as a feature. After binding, its color can be changed from brown to blue, the maximum absorbance from 470 nm into 595 nm.

The advantages of this method is simple and quick, and has highly sensitive, the disadvantage is vulnerable to the interference of salts.

First, took 50 µl Bio-Rad protein assay reagent and added 200 µl of protein samples, with 0.5 mg / ml BSA and ddH2O preparation for the final concentration 0、0.01、0.02、0.03、0.04、0.05 mg/ml be a standard solution (Table 2-1),

Samples of protein solution diluted 200 times with ddH2O, after average mixing, took 200µl to the 96 MicroWell™ Plates, then determined with 595 nm.

Table 2-1

Standard(part 1)

BSA (0.5 mg/ml)	0μl	5μl	10μl	15μl	20μl	25μl
ddH2O	200μl	195μl	190μl	185μl	180μl	175μl
Bio-Rad	50μl	50μl	50μl	50μl	50μl	50μl
Total	250μl	250μl	250μl	250μl	250μl	250μl
Final Concentration	0 mg/ml	0.01 mg/ml	0.02 mg/ml	0.03 mg/ml	0.04 mg/ml	0.05 mg/ml

Sample

sample protein	1.25
ddH2O	198.75μl
Bio-Rad	50μl
Total	250μl
diluted factor	200

2-10 Western blotting

Taking 30-50 μg protein samples to mix with 5x protein loading dye, with 95℃ heating for 5 min to process the SDS-PAGE electrophoresis.

3.75 % for stacking gel, 10 % or 12 % for separating gel. Loading the sample protein to the wells of the gel and running with 75 volts for 2.5hr, after finished electrophoresis, took out the gel to process the proteins translation. From the positive to negative in order to place Whatmam 3M filter paper, PVDF membrane, gel and Whatmam 3M filter paper. PVDF membranes to be pre-soaked in methanol, air bubbles should be out before it was fitted into the transfer holder. Placed into the transfer tank (Bio-Rad) and filling the transfer buffer, on ice for 2.5 hours with 100 volts to transfer

for getting the PVDF membranes. And then mixed the blocking buffer (5% skim milk in TBST) at room temperature for 1 hour. the primary antibody was diluted with TBST to 1:1000 and hybridized with the protein on PVDF membrane at 4℃ for overnight. Wash the membrane with TBST for 10 min for three times. The secondary antibody was diluted with TBST to 1:5000 and hybridized with the protein at room temperature for 1 hour, add cold reagent was observed after the machine recorded. Another work to do was to dilute the secondary antibody with TBST to 1:2000~5000 mixed at the room temperature for one hour and then added in the luminescence detection for observation and recordation under the machine.

2-11 Statistical analysis

We used the one way ANOVA to calculate the differences between groups, and post-tested with Neuman-Keuls test, when the P value of less than 0.05, as a statistically significant difference. The Data were expressed as mean ± SD.

Chapter 3. Result

3-1. The effect of electro-acupuncture on Taichong point for spontaneous hypertensive rats

We measured tail-pressures of the rats after adapted for 1-2 weeks. All the systolic, mean and diastolic blood pressure (BPs, BPm and BPd) of the SHR rats were significantly higher than the WKYs ($P<0.001$) (Table 3-1A).

At the 3rd day of therapy, all the BPs, BPm and BPd of EAT rats were reduced more than 15 mmHg, and significantly lower than the SHR rats ($P<0.001$), but there was no difference between SHR and EAS rats. All the BPs, BPm and BPd of EAT rats were also significantly lower than the rats of sham-point group ($P<0.05$, $P<0.01$, $P<0.05$) (Table 3-1B, Fig 3-1A).

At the 19th day of therapy, BPs of the SHR and EAS rats was increased to 188 mmHg. All the BPs, BPm and BPd of EAT rats were higher than those at the 3rd day, but BPs of EAT rats was still significantly lower than the SHR and EAS rats ($P<0.01$ and $P<0.05$), whereas the BPm and BPd of EAT rats were not different from the other two group (Table 3-1C, Fig 3-1B).

3-2. Histological analysis

All the rats were sacrificed after treated for 3 weeks. The hearts of each group were immersed in 10% formalin and sent to the Changhua Christian Hospital to cut and stain the tissue sections with Hemotoxylin and eosin (H & E) stain and Masson's Trichrome stain. The cardiomyocytes of the SHR and EAS rats were larger and more disarrayed than WKY. It was showed that the hearts of SHR and EAS rats were remodeling. After 3 weeks of electro-acupuncture on LR-3, the size and arrangement of EAT cardiomyocytes were reversed. This result supported that EA on Taichong point could avoid cardiac hypertrophy and remodeling caused by hypertension (Fig 3-2A).

The cardiomyocytes of SHR and EAS in trichrome stain showed more blue

stain of collagen aggregation (Fig 3-2B, the arrows) than WKY. After 3 weeks of electro-acupuncture on Taichong, the stain was almost disappeared in the LV section of EAT. This was shown that EA Taichong point can inhibit myocardial fibrosis caused by hypertension.

Furthermore, in the TUNEL assay, apoptotic reaction was significantly increased in LV of EAS and SHR rats (Fig 3-3A). After 3 weeks of electro-acupuncture on Taichong, TUNEL spots were significantly less in LV of EAT rats than SHR and EAS rats ($P<0.05$) (Fig 3-3A and B) which shown that EA Taichong point can inhibit myocardial apoptosis caused by high blood pressure.

3-3. Western blotting

(1) EA enhanced antioxidant and detoxification capacity

In left ventricular (LV) tissue of the SHR and EAS rats, protein level of SOD 1 was lower than that of WKY rats. It showed that the hypertension will reduce the heart's resistance to oxidative damage. After three weeks of acupuncture treatment on LR-3 point on SHR rats, SOD 1 was significantly increased in LV of the EAT rats (Fig 16). Even though eNOS was reduced slightly in LV of the hypertension rats, the active-form pNOS3 was obviously decreased in their heart. However, after EA on LR-3 for 3 weeks, both eNOS and pNOS3 were significant increased in LV of the EAT rats (Fig 3-4). The results supported that EA on LR-3 could enhance the capacity of anti-oxidant and mediating for cardiac functions.

(2) EA reduced the cardiac hypertrophic markers which were induced by hypertension

Hypertension led to increasing of inflammation markers like IL-6 in LV of SHR, and induced the raise of its down-stream hypertrophy marker STAT 3. After 3 weeks of EA on Taichong point, both IL-6 and STAT 3 in LV of EAT rats were lower than SHR and EAS. Another common cardiac hypertrophic marker BNP was shown in a similar tendency (Fig 3-5A and B).

(3) EA reversed associated proteins of apoptotic pathways

In the LV tissues of SHR and EAS rats, the death receptor FAS and its associated protein FADD were obviously increased than those in WKY but reserved in EAT (Fig 3-6A and B).

In another apoptotic pathway, more pro-apoptotic Bcl-2 family member Bax was found in LV of SHR and EAS than WKY, and so was the downstream protein Cytochrome c. But both Bax and Cytochrome c were decreased in LV of EAT (Fig 3-7A and B).

In another cardiac apoptotic pathway, IGF-ⅡR dependent apoptotic pathway, the associated proteins Gαq and Calcineurin were increased in LV of SHR and EAS and more than in WKY. But both the two proteins were decreased in LV of EAT (Fig 3-8A and B).

All the findings above suggested that electro-acupuncture on Taichong could inhibit cardiac apoptosis in hypertension rats.

Chapter4. Discussion and conclusion

4-1. The effect on blood pressure by electroacupuncture with LR-3 point

Apoptosis is a kind of gene regulated by the orderly, active, non-inflammatory cell death which is due to pathological or physiological stimulation[58].Under normal circumstances, mature individuals of cardiomyocyte apoptosis is rare, but when the heart is in a pathological state, such as cardiac overload or myocardial ischemia etc. , myocardial apoptosis will be occurred [13]

We explored the hypertension-induced myocardial damaged and some of proteins expression.

The effect on blood pressure by electroacupuncture with LR-3 point electroacupuncture with LR-3 has a short-term effect for lowing blood pressure. However, continuing stimulating to 19 days, its effect for lowing blood pressure was reduced.

In recent years, some of researchers [59, 60] have suspected the effect of antihypertensive efficacy with acupuncture.

The results of our experience was showed that the short term of acupuncture LR-3 can significantly reduce systolic blood pressure in spontaneously hypertensive rats. However, we need to take a further research about why long-term stimulated on the acupoint could cause less effective result.

4-2. Tissue sections from the heart to study the effect of protection by EA LR-3 Point

Hypertension can cause adverse effects on the heart, including hypertrophy, apoptosis and fibrosis[61]. According to our experimental research by, H & E staining, it can be observed that, the myocardial cell hypertrophy and disarrayed phenomenon in SHR and the EAS group (Fig 3-2A).

Trichrome staining in sections can be observed that the EAS group and SHR rats' heart have collagen aggregation phenomenon (Fig 3-2B), that was

shown the possibility of local fibrosis.

It also can be observed in the TUNEL assay, the EAS and SHR's cardiac cells showed that the proportion of DNA fragmentation was significantly higher than WKY (Fig 3-3) , this was Shown they have more vigorous role in apoptosis. However, after 7 days EA Taichong Point treatment, the heart of EAT group, the morphology, arrangement, collagen aggregation, DNA fragmentations situation was significantly improved, this was indicating EA Taichong Point can protect the heart from hypertension

4-3 Western blotting from the heart to study the effect of protection by EA LR-3 Point

It is an effective method to use molecular biological screening to study the pathogenesis of essential hypertension[62]. Clinical studies have shown that acupuncture on LR-3 is effective for lowing hypertension[63]. Puncture Taichong Point may reduce may reduce the SHR plasma by ET - 1, increased Serum levels of NO play an antihypertensive effect[64]. Endothelin was found by Japanese scholar YANAGISAWA in 1988[65] it has a strong effect for small molecules because it can cause a variety of blood vessels contraction. Some research[66]confirmed elevated plasma endothelia in essential hypertension really play an important role.

Superoxide dismutase(SOD) enzymes are groups of antioxidant enzymes. They are important antioxidant defense in nearly all cells exposed to oxygen. SOD is known to reverse liver fibrosis, it perhaps through reversion of myofibroblasts back to fibroblasts.[67] Human endothelial nitric oxide synthase (NOS3) gene has been linked to vascular endothelial cell (EC) dysfunction[68]. IL-6 acts as both a pro-inflammatory and anti-inflammatory. IL-6 plays an important role in the regulation of many immune functions , for example, activation of macrophages, B cell development, respond to inflammation, hematopoiesis function and acute reactions. Closely related to coronary artery disease and inflammation, inflammation-related

substances in blood such as acute phase proteins and cytokines, increase in the concentration will get more risks of coronary heart disease in patients[69].

We took further observation by Western blotting to see the molecular pathway how to affect the heart.

At the Beginning of the experiment, we could see the proteins of SOD and eNOS and Phosphorylation status (pNOS3) have dropped in SHR and EAS groups. After three weeks of treatment, the concentration of these proteins have increased. Downstream of IL--6 hypertrophy marker stat3 in SHR and EAS groups were decreased.

Japanese scientists Mukkoyama found that BNP secreted by the cardiac hormone, mainly secreted position is in the ventricular[70]

Heart failure has a high mortality and it is not easy to be diagnosed. BNP is valuable clinical significance in the diagnosis of heart failure[71].

we examined the hypertrophy marker BNP. It was decreased as well. All of data has shown electroacupuncture LR-3 does work on preventing heart failure from hypertension. Over expression of Fas suggest that apoptosis and Fas is involved in the myocardium ischemia-injury[72]. Fas-Associated protein with Death Domain (FADD) is the importance protein in early T cell development[73]. FADD has been shown to interact with Fas receptor [59, 60].

We checked the FAS and FADD in SHR and EAS group were high at beginning. After 3 weeks treatment, all of them were decreased.

Bax is a pro-apoptotic protein, increased levels of Bax protein can promote apoptosis. Through mitochondrial stress-induced, Bax plays a key role in apoptosis .

Cytochrome c is involved in the initiation of apoptosis. It releases to the cytoplasm, the protein binds apoptotic protease activating factor.

In mitochondria, Bax and Cytochrome c in SHR and EAS groups were increased. After 3 weeks treatment, both of them were decrease.

Cardiac-directed over expression of Gαq could result in left ventricular dysfunction[28].

Calcineurin plays an important role in keeping normal physical structure and function of cardiovascular system[74].

We found that Gαq and calcineurin in the IGF2-R dependent's apoptosis pathway were increased in SHR and EAS groups. However, after 3 weeks treatment , both of them were decreased as well($p<0.05$) This experiment confirmed that the electroacupuncture can protect .the heart from apoptosis.

Part Ⅱ

Comparing the Cardio-protective Effects of Catgut Embedding and Electro Acupuncture Therapy on TaiChong point in Spontaneous Hypertensive Rat

比較穴位埋線與電針太衝穴對高血壓大鼠的心臟保護效果

Chapter 5. Introduction

5-1 Catgut embedding therapy

Catgut embedding treatment for hypertension is taking advantage of gut tissue to keep continuing stimulation and its effect usually last longer more than ordinary acupuncture[13].

Acupoint catgut embedding therapy is fix the catgut into the acu-point , this purpose is through the acupuncture point stimulation to balance the yin and yang ,let the central nervous system and endocrine disorders can be restored , the whole body to circulation can be smoothly. then the blood pressure will be stably[58].

According to above theories, we have chosen LR-3 to treatment.

Su Wen said that: "Dizziness cause by wind belongs to the liver". TCM believe that the original etiology of the hypertension is from the liver, or excessive yang, hyperactivity yang; or depressive anger to damage the liver yin and blood. Liver and kidney yin deficiency, kidney essence insufficiency can cause meridians and collaterals obstruction and then myocardial disease will be happen.

LR-3 point belongs the yuan-primary point of the liver.

The book of Ling Shu Jiu Zhen Shi Er Yuan said: If five internal organs have sick , we should choose LR-3 to reduce its excess and tonify its deficiency to make yin and yang balance, to let the stagnation liver qi can smoothly in our body and to improve the symptom of hypertension.

5-2 The role of Insulin-like growth factor in myocardial cells

IGF-I and IGF-II play an important role in the cell growth and tissue differentiation. These factors in specific tissues and organs function are very important, Such as IGF-I, IGF-II to maintain the respiratory system, immune system and normal function of the cardiovascular system. In addition IGF-I[75] , IGF-II[76] in fetal growth and development also have important

functions, such as during cardiac development, however, only IGF-I still exists after birth , IGF-II can not be detected, it belongs to a Embryonic genes.

Many studies have shown that IGF-I prevents cardiomyocyte apoptosis and ventricular dilation [77] ; Some induced heart failure experiments also pointed out that [78, 79] IGF-I can prevent cardiomyocyte apoptosis and increased the function of heart constrictionTherefore, IGF-I is considered to be anti-apoptotic factor. IGF-I can inhibit apoptosis of myocardial cells is related with anti-apoptotic Bcl-2 family proteins (such as Bcl-2, Bclx) expression was increased . IGF-I can achieve the effect of inhibition of apoptosis is depend on the expression of anti-apoptotic Bcl-2 family proteins [80, 81].Those involved in the prevention of apoptosis signaling pathways, including tyrosine kinase、PI3 kinase、MAP kinase. PI3 kinase can make Akt phosphorylation, pAkt will attached to the mitochondrial phosphorylation on Bad, Bad release from the mitochondria into the cytoplasm, to maintain the integrity of mitochondrial membrane potential and to achieve the inhibition of apoptosis [82] (Fig 5-1).

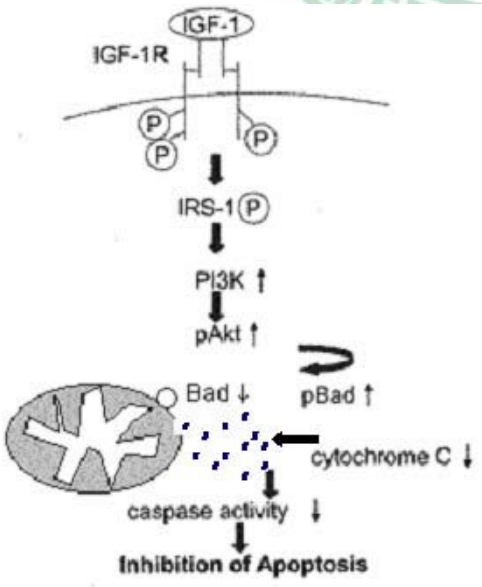


Fig5-1. IGF-1R dependent cardiac survival pathway[83].

Chapter 6. Materials and Methods

6-1. Source of experimental animals

8-week-old male SHR (Spontaneously hypertensive rats / Charles River Laboratories, SHR / NCrlNarl) rats and 8-week-old male WKY rats (Wistar-Kyoto, WKY / CrlBltw) were purchased from the National Science Council of the National Laboratory Animal Center. The rats housed at a controlled ambient temperature of 21-23℃ with 50 %-60 % relative humidity and a 12 hr/12 hr light (9:00-21:00)/ dark (21:00-9:00) cycle. The rats were fed with pelleted rat chow and RO water at libitum. After the adaptation for 1-2 weeks, we measured their tail pressure separately to make sure that blood pressure of the SHRs was significantly higher than WKY rats, and then went on the next steps to group them for the follow-up experiment.

6-2 Experimental animal groups

1. WKY: WKY male rats, as the negative control group with normal blood pressure
2. SHR: SHR male rats with sp ontaneous hypertension as a positive control group
3. EAS: SHR male rats for acupuncture treatment on sham-points
4. EAT: SHR male rats for acupuncture treatment on Taichong Point
5. CES: SHR male rats for catgut embedding treatment on the sham-points
6. CET: SHR male rats for catgut embedding treatment on Taichong points

6-3 the animal experiments

After adaption for 1-2 weeks, we randomized them into the six groups including the WKY group (WKY), the SHR untreated group (SHR), electro-acupuncture sham-point group (EAS), electro-acupuncture treatment group (EAT), catgut-embedding sham-point group (CES), and catgut-embedding treatment group (CET). For treating the rats, they were

anesthetized with isoflurane, and then we punctured them in the pseudo-points or Taichong point (LR-3) with electrical/catgut stimulation. The EA groups were treated once per day and the CE groups were treated at the 1st and 4th day. We measured their tail pressure at the 0, 3rd and 7th day. After the treatment for a week, all the rats were sacrificed, and their hearts were harvested. One of each group was put in 10 % formalin and the others were stored in -80 ℃.

6-4 The treatment

6-4-1. Locations of treatment points

A. Taichong point: It locates on the dorsum of the foot, between the first and second metatarsal bones, in the depression anterior to the combining site of the base of metatarsal bones. Depth of about 2-4 mm

B. Sham-Point: We chose the point was on the foot dorsal, between the third and fourth metatarsal depression in front of the bottom junction, depth of about 2-4 mm.

6-4-2. Electro-acupuncture parameters

The rats were quickly anesthetized with 5 % isoflurane mixed with oxygen, and then changed to 2-2.5 % to maintain anesthesia. Before puncture, we sterilized the local region with 75 % alcohol, inserted the needle of 32 G * 0.5-inch size respectively, and then connected to the positive and negative electrodes, and followed the parameter settings: 2 Hz / 1 mA/150 ms, 30 minutes has been set for every treatment per day.

6-4-3. Catgut-embedding parameters

We used 24G * 1 inch injection needle be a outer needle I and 30G * 1.5 inch acupuncture needle be an inner needle, and about 0.5 cm 4/0 catgut immersed in 75% alcohol for use. The rats were quickly anesthetized with 5 % isoflurane mixed with oxygen, and then changed to 2-2.5 % to maintain

anesthesia. We embedded the absorbable catgut on the Taichong point (LR-3) or sham-point of the rats. The method of embedding as following: insert the acupuncture needle into the matched injector then stuff with matched size of the catgut, and then puncture the needle points to the muscle layer, and make sure the catgut was completely embedded. The CE groups were treated at the 1st day and 4th day.

6-5 stained tissue sections

After sacrificed, we washed out the blood with cold PBS and got their heart tissue, then soaked in 10 % (w/v) formaldehyde at least 24 hours. The heart samples were sent to Changhua Christian Hospital to have embedded, sliced, and analyzed with Hematoxylin and Eosin stain (H & E stain) and Masson's Trichrome stain. H & E stain and Trichrome stain of the biopsies were observed and recorded with 400 times of the microscope (Olympus).

6-6. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL assay)

Sections were incubated in the hybridization incubator at 58℃ for overnight. Next day, sections were put into 100% Xylene for 5 min (repeat thee times), and then put in 100 %, 95 %, 90 %, 85 % and 75 % alcohol each for 5 minutes sequentially, and finally washed into ddH2O repeatedly twice for 5 minutes. This process was for dewaxing and rehydration. Removed the slices and wipe the slices around with lens tissue around carefully (don’t let the slices dry out). Use the DaKo pen to fence the scope of the sections. We covered the sections with proteinase K for 30 min in dark (range of coverage can be sliced off), then washed in PBS for 5 minutes twice. Added 3 % H2O2 for 10 minutes and washed with PBS for 5 minutes twice. Reacted with the permeabilisation solution (0.1 % sodium citrate) for 8 minutes and repeated washing step. Reacted with the blocking buffer for 1 hour, and washed with PBS for 5 minutes twice. Reacted with the mixture of the

Enzyme Solution and Label solution (in 1:9 ratio) (In Situ Cell Death Detection Kit, 11684817910, Roche, Mannheim, Germany) in dark for 1 hour at 37 ℃, and then washed with PBS for 5 minutes twice. Reacted with DAPI stain (D9564, Sigma, USA) diluted to 20,000 times by PBS in dark for 5 min, and then washed with PBS for 5 minutes twice. Finally, the sections were observed and recorded with the fluorescent microscope.

6-7. process of disengaging for the left ventricle

After the animal models sacrificed, took their heart tissues and soaked in the icy PBS and then washed out blood, moved off the blood vessels which attached to the organs, also clipped the fat tissues and other connective tissues and then made them in dried condition. Cut away the left and right atrium and right ventricle. Finally, divided the disengaged left ventricle into equal portions, the weight for each part was about 0.1 g. All of them were put into centrifuge tubes and stored in -80℃.

6-8. extracting the protein of LV

Got 0.1 grams of left ventricular tissue, added 1 mL homogenization buffer (20mM Tris, 2mM EDTA, 10 % glycerol, 50 mM 2-mercaptoethanol, protease inhibitor, 1/1000 phosphatase inhibitor, pH7.4), put on the ice, ground by tissue homogenizer, Centrifuged and rolled for 12,000 rpm at 4 ℃ for 40 minutes and repeated above for wice, then stored in -80℃ for overnight, Repeated above once again and then got supernatant fluid. This is the tissue protein solution.

6-9. Bradford protein assay

The quantitative concentration of protein we used Bradford protein assay. The basic principle is according to Coomassie Brilliant Blue G-250 can easily bind protein as a feature. After binding , its color can be changed from brown to blue, the maximum absorbance from 470 nm into 595 nm.

The advantages of this method is simple and quick, and has highly sensitive, the disadvantage is vulnerable to the interference of salts.

First, took 50μl Bio-Rad protein assay reagent and added 200μl of protein samples, with 0.5 mg / ml BSA and ddH2O preparation for the final concentration 0、0.01、0.02、0.03、0.04、0.05 mg/ml be a standard solution (Table 3),

Samples of protein solution diluted 200 times with ddH2O, after average mixing, took 200μl to the 96 96 MicroWell™ Plates, then determined with 595 nm.

Table.6-1. Standard solution(part2)

BSA (0.5 mg/ml)	0μl	5μl	10μl	15μl	20μl	25μl
ddH2O	200μl	195μl	190μl	185μl	180μl	175μl
Bio-Rad	50μl	50μl	50μl	50μl	50μl	50μl
Total	250μl	250μl	250μl	250μl	250μl	250μl
Final Concentration	0 mg/ml	0.01 mg/ml	0.02 mg/ml	0.03 mg/ml	0.04 mg/ml	0.05 mg/ml

Sample

sample protein	1.25
ddH2O	198.75μl
Bio-Rad	50μl
Total	250μl
diluted factor	200

6-10 Western blotting

Taking 30-50μg protein samples to mix with 5x protein loading dye, with 95℃ heating for 5 min to process the SDS-PAGE electrophoresis.

3.75% of the upper stacking gel, 10% or 12% separating gel at the lower part. Loading the sample protein to the wells of the gel and running with 75 volts for 2.5hr, after finished electrophoresis, took out the gel to process the proteins translation. From the positive to negative in order to place Whatmam 3M filter paper, PVDF membrane, gel and Whatmam 3M filter paper. PVDF membranes to be pre-soaked in methanol, air bubbles should be out before it was fitted into the transfer holder. Placed into the transfer tank (Bio-Rad) and filling the transfer buffer, on ice for 2.5 hours with 100 volts to transfer for getting the PVDF membranes. And then mixed the blocking buffer (5% skim milk in TBST) at room temperature for 1 hour. the primary antibody was diluted with TBST to 1:1000 and hybridized with the protein on PVDF membrane at 4℃ for overnight. Wash the membrane with TBST for 10 min for three times. The secondary antibody was diluted with TBST to 1:5000 and hybridized with the protein at room temperature for 1 hour, add cold reagent was observed after the machine recorded. Another work to do was to dilute the secondary antibody with TBST to 1:2000~5000 mixed at the room temperature for one hour and then added in the luminescence detection for observation and recordation under the machine.

6-11 Statistical analysis

Used the one way ANOVA to calculate the differences between groups, and post-tested with Neuman-Keuls test, when the P value of less than 0.05, as a statistically significant difference

Chapter 7. Results

7-1. The effect for reducing blood pressure by EA/CE

To make sure the SHRs were with hypertension, we measured tail-pressures of the rats after adapted for 1-2 weeks. All the systolic, mean and diastolic blood pressure (BPs, BPm and BPd) of the SHRs were significantly higher than the WKYs ($P<0.001$) (Table 2A).

At the 3rd day of therapy, all the BPs, BPm and BPd of EAT rats were reduced more than 15 mmHg, and significantly lower than the SHR rats ($P<0.001$) (Table 2B). The similar effect was shown on CET rats, and all the blood pressures of CET rats was slightly higher than EAT rats but not with significance. There was no difference between SHR and both the two sham group (EAS and CES). Like EAT rats, the blood pressures of CET rats were significantly lower than CES rats except BPs ($P>0.05$, $P<0.05$, $P<0.05$) (Table 2B).

At the 7th day of therapy, the blood pressures were continuatively reduced by both electro-acupuncture and catgut-embedding. The average BPs, BPm, and BPd of CET rats were 133.50, 98.17 and, 80.39 mmHg, overtaking those of EAT rats (139.28, 101.56, and 83.56 mmHg) (Table 2C), but there was no significant difference between these two group.

7-2. Histological analysis

As showed in Fig 8A, Cardiac hypertrophy and disarray caused by hypertension can be reversed by stimulation of Taichong point with both electro-acupuncture and catgut-embedding for a week.

Left ventricular of the SHR rat stained by trichrome showed more blue stain of collagen aggregation than the WKY rat, and so did the EAS and CES rats (Fig 8B). After treatment of Taichong with EA/CE for a week, the stain was almost disappeared in LV sections of the EAT and CET rats.

Tissue sections of the rat hearts were stained by TUNEL assay. The

apoptotic nuclei were identified by the characteristic fragmented nuclei. The nuclear location was confirmed with DAPI stain. As shown in Fig 23A, the TUNEL spots were obviously much more in hypertension rats (SHR, EAS, and CES) than the healthy rat (WKY). After a week of therapy with EA/CE on LR-3, the apoptotic cells were significantly fewer in the hearts of EAT and CET rats. The statistical analysis was shown in Fig 23B.

All the histological phenomenon supported that EA/CE on LR-3 could protect heart from injury by hypertension, like remodeling, fibrosis and apoptosis.

7-3. Western blotting

7-3-1. SOD was increased much more in LV of CET rats than EAT rats.

The SOD 1 protein expression in SHR left ventricle volume was lower than the WKY，After treated with electro-acupuncture has rebounded，CET group even higher than that of WKY and EAT groups and achieve significant differences (Fig 12A and B).

7-3-2. EA/CE reduced the cardiac hypertrophy markers induced by hypertension

Some reports supported [3] that cardiac hypertrophy would cause inflammation. In our experiment, We found more TNFα in LV of the hypertension rats than the normal rats by Western blotting(Fig 7-5A). After one week of therapy, TNFα was decreased in LV of all the therapy groups (including sham-point and LR3), among of them, EAT group showed the best effect. We also found another inflammation factor IL-6 and its down-stream hypertrophy marker STAT 3 increased in LV of SHR, EAS, and CES rats. After EA/CE for a week, both IL-6 and STAT 3 were decreased in LV of EAT and CET rats. The effect of catgut embedding therapy is better than electro-acupuncture, but there was no significant difference between these two groups.

7-3-3. EA/CE reduced the cardiac apoptotic markers induced by hypertension

To make sure whether hypertension could induce apoptosis of myocardium, western blot was performed for detecting Bcl-2 and Bax in LV of the rats. We found that hypertension caused decrease of the anti-apoptotic marker Bcl-2, but this result could be reversed by EA/CE for a week. The pro-apoptotic marker Bax showed a opposite tendency from Bcl-2 (Fig 14).

In another cardiac-apoptotic pathway, IGF-2R dependent apoptotic pathway, we also found that the associated protein PLCβ3 and pPKCα were raised in LV of SHR, EAS and EAT rats. After one week of therapy, The EATgroup to pPKCα has a more significant impact, but the CET group to PLCβ3 has a more significant impact (Fig 15 A and B).

7-3-4. Stimulation on LR3 would elevate protein expression of cardiac survival markers which were reduced by hypertension.

IGF-1R dependent survival pathway is an important mechanism against apoptosis in cardiomyocytes. To clarify whether EA and CE would inhibit apoptosis by activating this IGF1-PI3K-Akt pathway, LV tissue were homogenized and measured by Western blot. All the associated markers like IGF-1Rα, PI3K, and the active-form pPI3K were reduced in LV of the hypertension rats (SHR, EAS and CES) (Fig 7-8A). After one week of treatment, all these proteins were increased. Furthermore, PI3K and pPI3K were much more in CET rats than EAT rats (Fig 7-8B). Akt, the downstream protein of PI3K, and the active-form pAktT308 were also reduced in hypertension rats but restored in the treated rats (Fig 7-9A and B).

8 Discussion and conclusion

8-1 To observed the protecting the heart of SHR by EAT and catgut embedding treatment (CET)on Taichong point

Dan Huang and Li-De Zhang investigated for past 5 years on hypertension be treated with acupuncture results, it has shown that acupuncture for treating high blood pressure was significant effect [84].

Fang Zuo and Ting Lou have Studied of 518 articles for catgut embedding, their conclusion is that the catgut embedding will have much wider use in future and need to be improved[85].

Effect by catgut embedding is a unique method, as in the acupoint catgut will turn to softening, decomposition, liquefaction and absorption process , and then the acu-points will get the physiological, physical and biochemical stimulation[86].

Cui-Ying Xu and Feng-Ling Wang found that injection Cuan xong (ligustrazine)in Taichong Acupoint for treating hypertension was an exact method[87].

There were three major be found in this study. First, from the result of H& E stain on heart tissue biopsy, we found that From heart tissue biopsywith TUNEL dyeing we observed that either the electracupuncture or catgut embedding therapy can suppressed some of apoptosis proteins from the heart tissue , and then detected by Western blot to check their pathways.

Apoptosis has made it clear there are three signal transduction pathways, death receptor pathway, mitochondrial pathway, endoplasmic reticulum signaling pathways[88]. The final way is through the activation of Caspases proteases achieved. A research has shown that removing PKC alpha can improved the hearts ability to contract[89]. Interleukin-6 (IL-6) is secreted by T cells and macrophages to stimulate immune response to foreign pathogen. IL-6 relevant to IL-6 is relevant to atherosclerosis process[90]. Epidemiological studies have revealed an association between inflammation and cardiovascular disease due to hypertension[15, 16]. In our research has

shown that compared with SHR and EAS group, the IL-6 in EAT group was significant decreased. That means acupuncture did work with Anti-inflammatory function.

The main representative of BCL2 family mediated by variety of pathways to apoptosis[91]. Akt regulates cellular survival and metabolism[92].

There was a research has shown that When hypertension in the third stage with complications of heart failure or cerebral infarction, the growth hormone or insulin growth factor-1 will lower than the normal person[93].

The phosphatidylinositol 3-kinase has a key regulatory function for many cellular processes[94]. Superoxide dismutases (SOD)have three forms in human. SOD1 binds copper and zinc ions. Lack of SOD1 will get a shortened lifespan[95]. Nitric oxide (NO) is an important regulator of blood pressure (BP) which produced by endothelial NO synthase (eNOS)[96]. eNOS can prevent enzyme to oxidative stress. Another proteins such as Bcl-2 family members Bax and Bak play a critical role in apoptosis[97].

The bcl2, IGF1-R, Akt, PI3K, SOD and eNOS protein can avoid cytochrome c be released from the mitochondrion. It means they can prevent caspase 3 be induced. We have found catgut embedding therapy(CET) or electroacupuncture therapy (EAT) can suppress the activation of Caspase 8 and Caspase 3 and also can avoid apoptosis while also can suppress the activation of death receptor-independence , apoptosis can be reduced though this process.

After treatment, the pro-apoptosis protein Bak of performance was decreased and anti-apoptosis apoptosis protein SOD, and IGF1-R, and Akt, and PI3K, and Bcl and eNOS were increased.

By Trichrome dyeing can be found either EAT or CET can reduced blue collagen protein fiber produced from the SHR's myocardial organization ., it was shown that the EAT or CET therapy can prevent the heart from fibrosis. According to Western blot analysis, we have found that the proteins of FAS 、FADD、 Bax、 Cytochrom C、 Gαq 、calcineurin 、PLCβ3、

PKCα can be suppressed and FAS,,FADD,Bax,Cytochrom C, G Alpha q, calcineurin, PLC Beta 3, and PKC Alpha protein of performance were decreased and IL-6, STAT3 and BNP were suppressed . the concentration of IL-6 and TNF alpha also decreased . At the same time we also found that both EAT or CET can increased bcl2, IGF1-R, Akt, PI3K, SOD and eNOS protein expression, that means EAT or CET on Taichong point can prevent apoptosis from hypertension.

8-2 To research the EA T/ CET Taichong Point on protecting the heart

Left ventricular hypertrophy is the major problem in patient who has hypertension[98].

The SHR group's myocardial cell got hypertrophy and disarrayed which phenomenon can be observed by the H & E staining ,that means its heart is hypertrophy and remodeling. The groups of sham-point (EAS, CES) also have remodeling situation. After EA / catgut embedding Taichong Point for a week , the shape and arrangement of myocardial cells have clear recovery , and similar with WKY. Trichrome stain also has the same result, SHR, EAS, CES groups have significant collagen aggregation but EAT and CET group did not. TUNEL assay showed that the hypertension will increase in myocardial apoptosis, and EA or catgut embedding on Taichong Point could inhibit apoptosis, the effect of catgut embedding has a little better than EA group (1.3%: 1.5%), but there were no significant difference between two groups (P> 0.05).

8-3 To determine the EA / catgut embedding in Taichong Point on the effect of protecting heart by Western blotting

We further observed by Western blotting for looking for the molecular pathway to see how the Taichong Point affect on the heart , It can be seen in Fig 7-4 SHR, EAS and the CES groups, their volume of SOD 1 in the left ventricular less than WKY, this is indicating a lower ability to resist

oxidative damage, and after a week of EA / embedding therapy, the amount of SOD 1 in EAT AND CET Rats have significantly increased in the left ventricular (Fig 7-4A), while the effect of catgut embedding group was better than EA group and achieve significant ($P < 0.001$, Fig 7-4B).

In the aspect of cardiac hypertrophy, the literature pointed out that the myocardial hypertrophy can company with inflammation and the release of TNFα, IL-6 and other cytokine [29]. In our research was found that the amount of TNFα in SHR's left ventricle was higher than WKY and up to 1.5 times (Fig 7.5B)，TNFα expression was reduced among l the EA / catgut embedding ,even sham-point as well, but EA Taichong Point was the best, but the effect of catgut embedding has slightly better than sham-point but not significant difference. Both EAT and catgut embedding sham-point can effectively reduce TNFα, it may be due to stimulation can induce primary immune system itself, The acupuncture Taichong points can further reduced TNFα performance, and achieve significant difference with the EAS group ($P<0.01$), (Fig 7-5B).

Another cytokine IL-6 and its downstream hypertrophy marker STAT 3 were increased in LV of SHR, EAS and CES rats. but after a week of treatment, EAT and the CET of the IL-6 and STAT 3 mRNA levels were decreased, catgut embedding group was better than EAT group but no significant, this is indicating the catgut embedding was little better than EAT group.

In the anti-apoptotic aspect , we examined the Bcl-2 family of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax. We found that hypertension would reduce Bcl-2 and increase Bax in left ventricular(LV) of the rats.

In the EAT group of Bcl-2 expression was increased slightly and better than catgut embedding group ,but in the catgut embedding group the Bax was better than EAT group, but there were no significant difference (Fig 7-6).

In another IGF-2R dependent apoptotic pathway, we also found that PLCβ3

and pPKCα were increased in LV of hypertension rats. Both of EA and catgut embedding groups can reduce the performance of these two proteins. EAT was better for pPKCα , but the catgut embedding was better for PLCβ3.

Hypertension (hypertension) is morbidity and risk factor for atherosclerosis (AS), cerebrovascular disease and also closely related to insulin resistance[99], obesity, obstructive Sleep apnea syndrome [100]and terminal kidney disease [101]. Myocardial cell apoptosis occurs are hypertension and the foundation of development of Cytology [102]. Apoptosis of myocardial cells are affected by a variety of cell signaling pathways controlling large amounts of myocardial cell apoptosis not only can lead to ventricular remodeling and cardiac arrhythmias, can also affect myocardial energy metabolism and contractility[103].

According to TCM theory, for treating hypertension, we pay more attention on the symptom differentiation such as LR-3 for excessive liver-yang syndrome[104], ST40 for phlegm damp obstruction[105], BL23 and KI-3 for kidney essence deficiency[105], ST36 for qi and blood deficiency[105].

Therapeutic effect of acupuncture treatment for hypertension is safe and non-toxic side effects. But clinical researches exist some problem, as effect standard, less of experimental research. Hypertension is a cardiac chronic medical condition .Our goal in future is keep studying forward to getting the best method for prevent complications from high blood pressure in the field of acupuncture.

Table 3-1. Compared the rats in each group after acupuncture treatment of changes in blood pressure

A.

Comparisons with BP of rats of at 0 day				
Group	No.	BPs	BPm	BPd
WKY	11	114.17 ± 16.93	83.49 ± 7.71	67.78 ± 9.86
SHR	18	169.54 ± 13.61***	131.62 ± 11.19***	112.44 ± 12.30***
ps. Compared with WKY, ***P<0.001.				

B.

Comparisons with BP of the rats at 3rd day				
Group	No.	BPs	BPm	BPd
WKY	9	125.24 ± 10.33	91.93 ± 8.96	75.33 ± 8.90
SHR	6	175.25 ± 4.41***	134.69 ± 2.68***	114.19 ± 4.64***
EAS	6	167.86 ± 7.34***	127.69 ± 8.14***	107.42 ± 10.91***
EAT	6	153.31 ± 9.75###△	108.67 ± 10.80###△△	86.42 ± 12.83###△
ps. Compared with WKY, ***P<0.001.				
Compared with SHR, ##P<0.01; ###P<0.001.				
Compared with EAS, △P<0.05; △△P<0.01.				

C.

Comparisons with BP of the rats at 19th day				
Group	No.	BPs	BPm	BPd
WKY	3	117.17 ± 10.77	81.67 ± 10.92	62.83 ± 12.79
SHR	3	188.75 ± 5.77***	139.75 ± 8.98**	115.17 ± 11.62**
EAS	3	188.83 ± 9.70***	141.83 ± 5.31***	118.25 ± 6.25***
EAT	3	168.92 ± 2.63##△	133.92 ± 6.00	116.25 ± 7.81
ps. Compared with WKY, **P<0.01; ***P<0.001.				
Compared with SHR, #P<0.05; ##P<0.01.				
Compared with EAS, △P<0.05.				

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong) for 3 weeks and measured the BPs, BPm, and BPd (systolic, mean, and diastolic blood pressure) with their tail. A) Baseline BP of the rats before treatment. B) BP of the rats at the 3rd day in treatment. C) BP of the rats at 19th day in treatment. (Data are expressed as mean ± SD)

Table 7-1. compared each group's rats blood pressure change situation after one week treatment by electro-acupuncture or catgut embedding therapy .

A.

Comparisons of HR and BP of rats of at 0 day				
Group	No.	BPs	BPm	BPd
WKY	11	114.17 ± 16.93	83.49 ± 7.71	67.78 ± 9.86
SHR	30	171.11 ± 12.01***	132.28 ± 11.11***	112.70 ± 12.47***
ps. Compared with WKY, ***P<0.001.				

B.

Comparisons with BP of the rats at 3th day				
Group	No.	BPs	BPm	BPd
WKY	9	125.24 ± 10.33	91.93 ± 8.96	75.33 ± 8.90
SHR	6	175.25 ± 4.41***	134.69 ± 2.68***	114.19 ± 4.64***
EAS	6	167.86 ± 7.34***	127.69 ± 8.14***	107.42 ± 10.91***
EAT	6	153.31 ± 9.75###△	108.67 ± 10.80###△△	86.42 ± 12.83###△
CES	6	169.14 ± 8.06***	129.06 ± 7.81***	108.75 ± 9.63***
CET	6	158.03 ± 11.43##	115.61 ± 9.01###§	94.06 ± 9.54###§
ps. Compared with WKY, ***P<0.001.				
Compared with SHR, ##P<0.01; ###P<0.001.				
Compared with EAS, △P<0.05; △△P<0.01.				
Compared with CES, §P<0.05.				

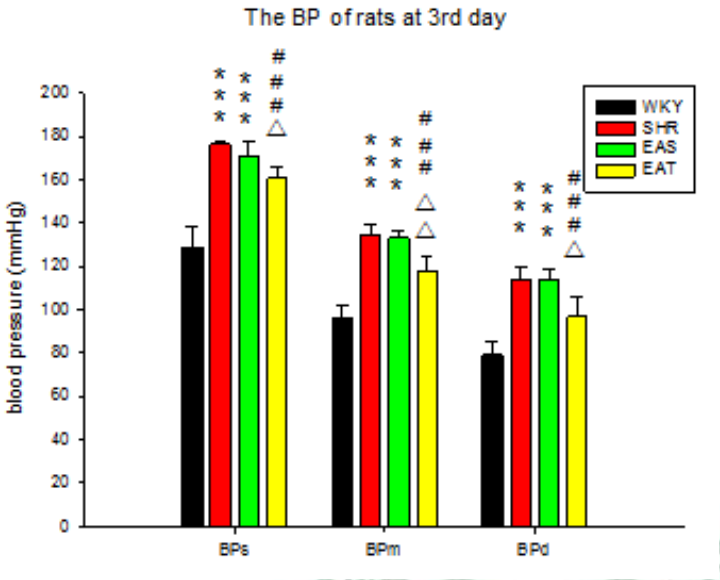
C.

Comparisons with BP of the rats at 7th day				
Group	No.	BPs	BPm	BPd
WKY	3	118.89 ± 11.79	81.33 ± 2.20	63.06 ± 1.80
SHR	3	173.73 ± 1.39***	132.54 ± 1.79***	111.87 ± 3.21***
EAS	3	166.28 ± 8.00***	124.44 ± 12.57***	103.33 ± 14.78**
EAT	3	139.28 ± 10.30##△	101.56 ± 3.67###△	83.56 ± 5.82##
CES	3	164.39 ± 9.48***	124.00 ± 13.37***	103.78 ± 15.39**
CET	3	133.50 ± 4.84###§§	98.17 ± 3.40###§	80.39 ± 5.87##
ps. Compared with WKY, **P<0.01; ***P<0.001.				
Compared with SHR, ##P<0.01; ###P<0.001.				
Compared with EAS, △P<0.05.				
Compared with CES, §P<0.05; §§P<0.01.				

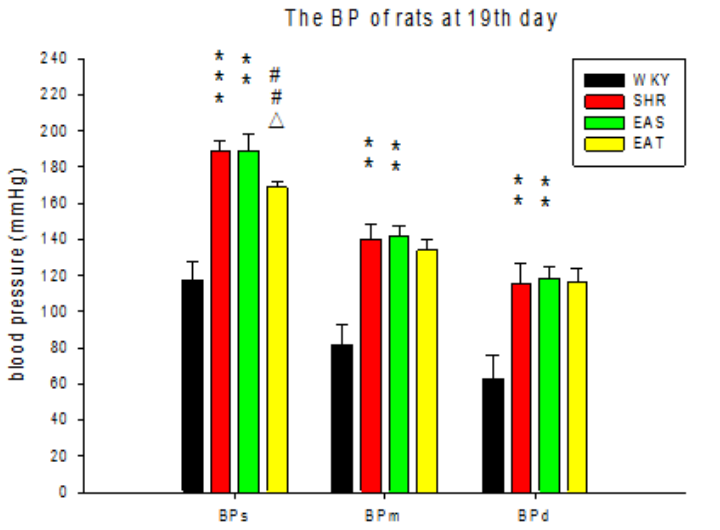
The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 3 weeks and measured the BPs, BPm, and BPd (systolic, mean, and diastolic blood pressure) with their tail. A) Baseline BP of the rats before treatment. B) BP of the rats at the 3rd day in treatment. C) BP of the rats at 19th day in treatment. (Data were expressed as mean ± SD)

Fig 3-1

A.


The BP of rats at 3rd day
blood pressure (mmHg)
0
20
40
60
80
100
120
140
160
180
200
BPs
BPm
BPd
WKY
SHR
EAS
EAT

B.


The BP of rats at 19th day
blood pressure (mmHg)
0
20
40
60
80
100
120
140
160
180
200
220
240
BPs
BPm
BPd
WKY
SHR
EAS
EAT

C.

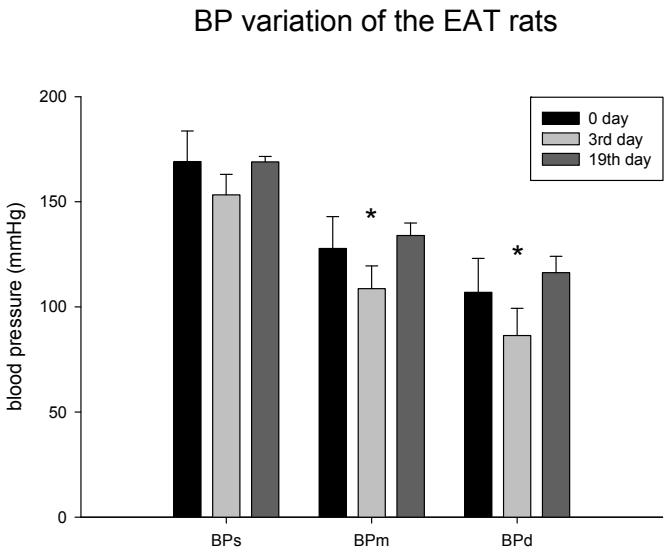
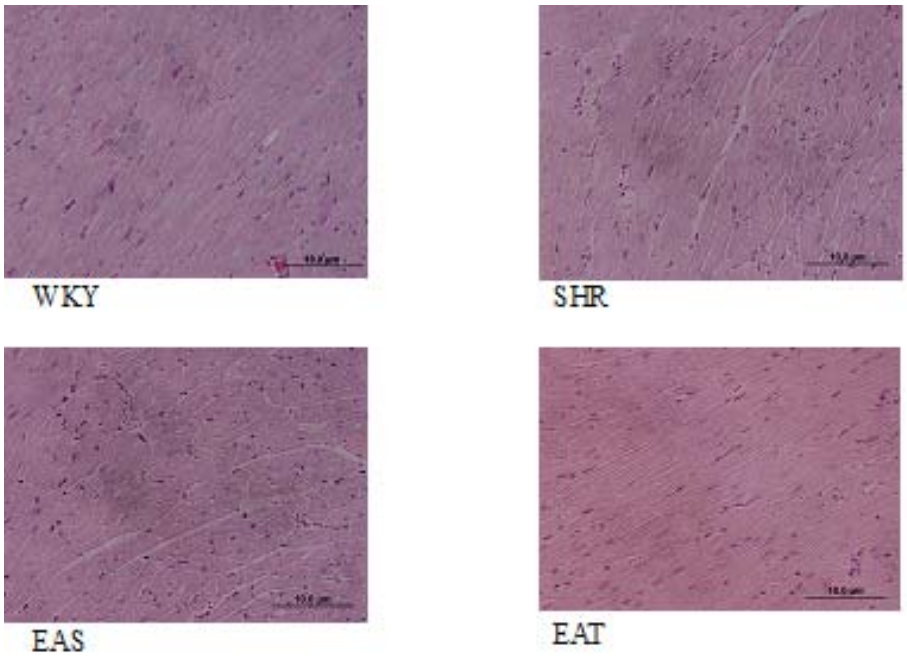


Fig 3-1. Comparing BP of the rats at 3rd and 19th day.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong.) for 3 weeks. A) The BP of the rats at 3rd day. B) The BP of the rats at 19th day. C) BP variation of the EAT rats in this 3-week treatment. (Compared with WKY, ***P<0.001. Compared with SHR, #P<0.05; ##P<0.01, ###P<0.001. Compared with EAS, △P<0.05, △△P<0.05 in Fig 3-1A and B. Compared with 0 day, *P<0.05 in Fig 3-1C.)

Fig 3-2

A.



B

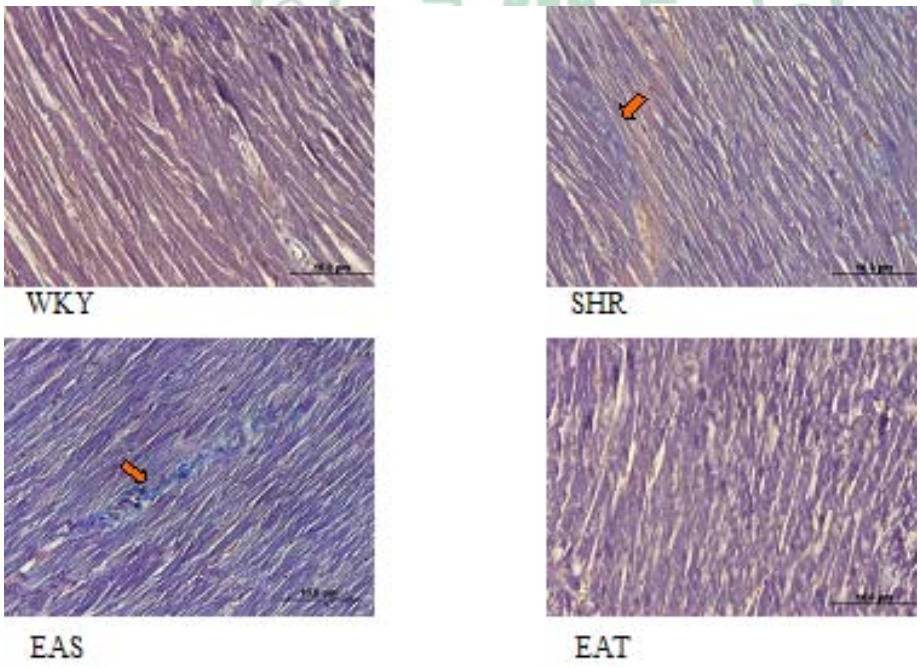
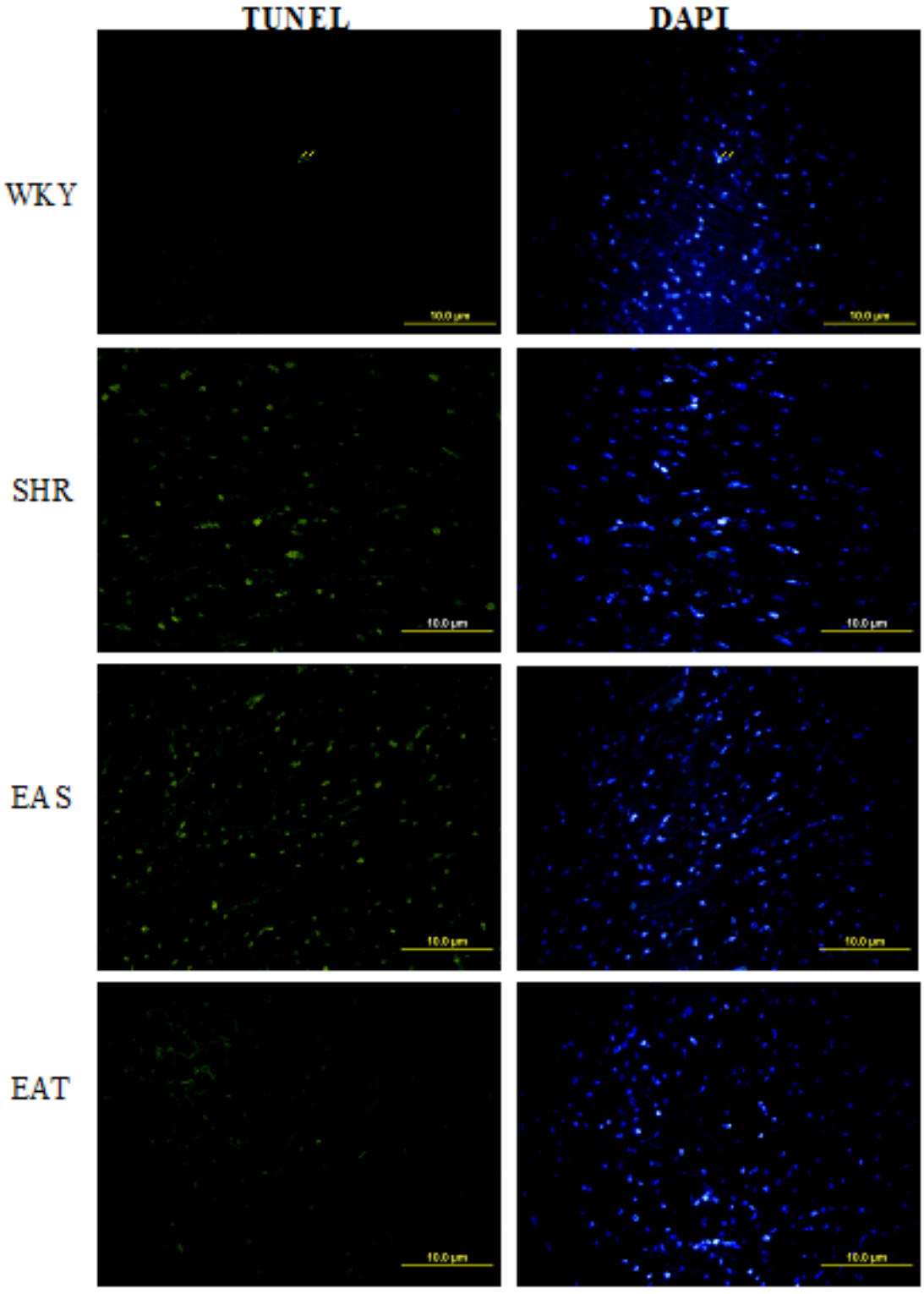


Fig 3-2. transverse sections of LV cardiomyocytes of the rats.

A) H&E staining (400X). The cardiomyocytes of SHR and EAS rats were larger and more disarrayed than WKY and EAT. B) Masson's trichrome staining (400X). The cardiomyocytes of SHR and EAS showed more blue stain of collagen aggregation (the arrows) than WKY and EAT.

Fig 3-3



B.

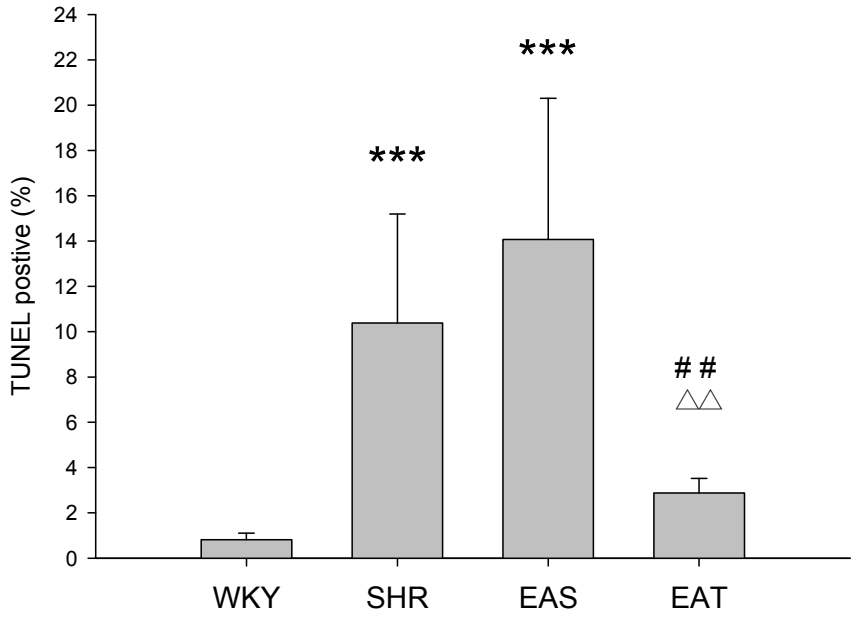
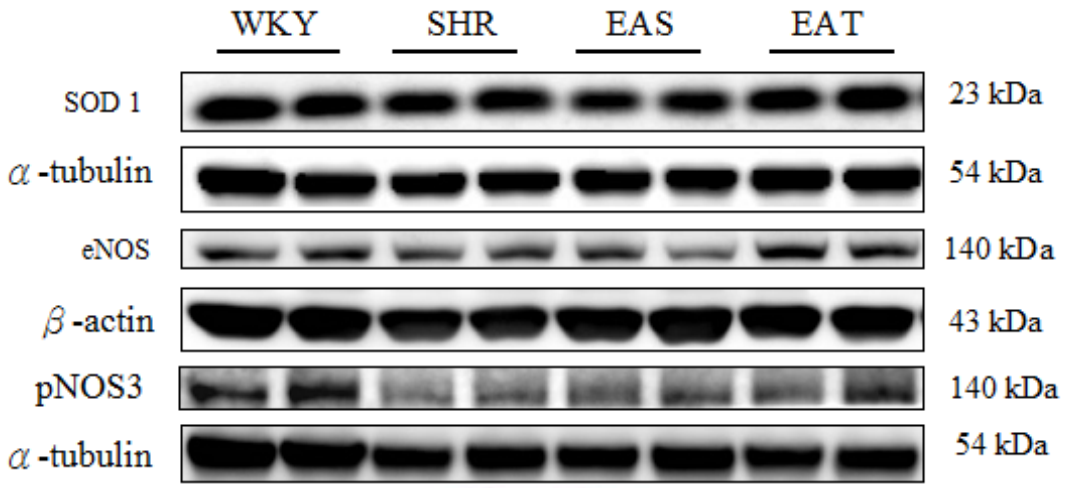


Fig 3-3. TUNEL assay of LV sections of the rats.

A) More TUNEL spots were detected in LV of SHR and EAS rats than WKY, and the LV section of EAT showed less TUNEL spots than SHR and EAS. B) stick plot of Fig 4A, the apoptosis percentage in cardiomyocytes of EAT heart was significantly lower than SHR and EAS. (***P<0.001, compared with WKY: ##P<0.01, compared with SHR; △△P<0.01, compared with EAS.)

Fig 3-4

A



B.

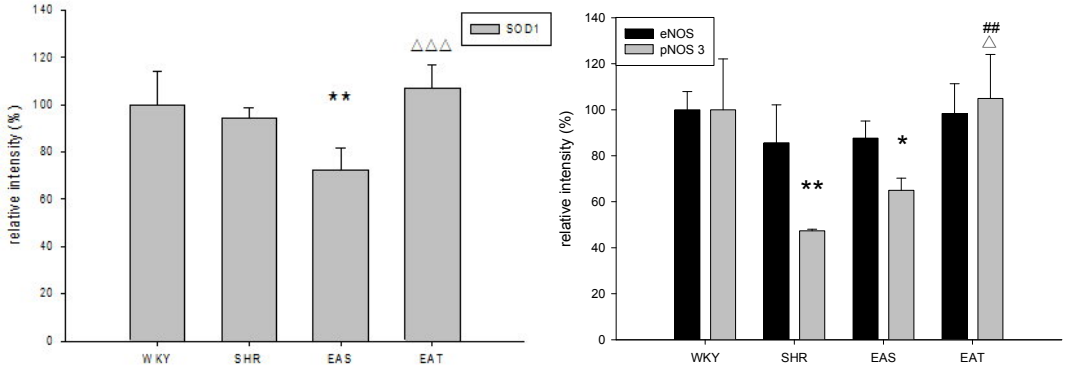
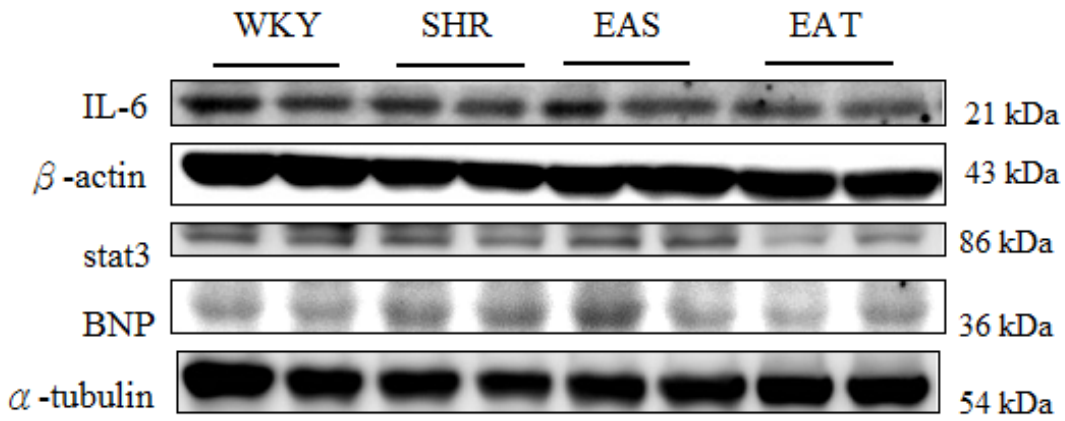


Fig 3-4. Protein levels of the SOD 1 and eNOS.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong) for 3 weeks and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA).Compared with WKY, **P<0.01. Compared with SHR, ##P<0.01. Compared with EAS, △△△P<0.001.

Fig 3-5

A.



B.

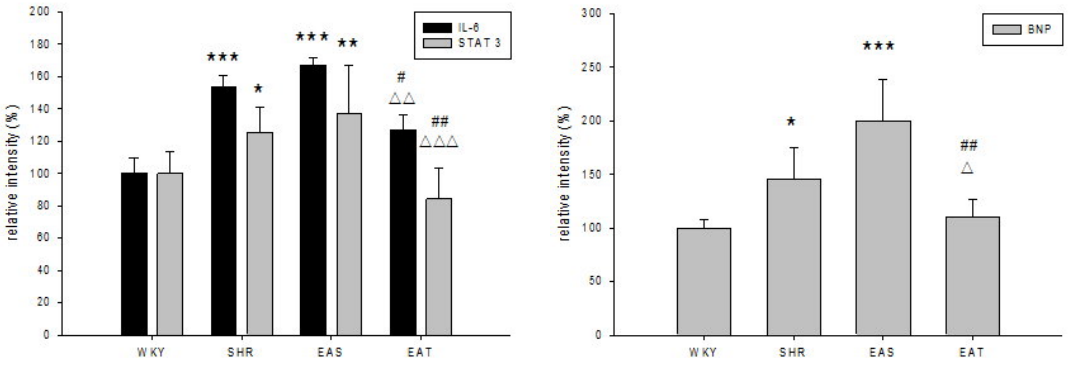
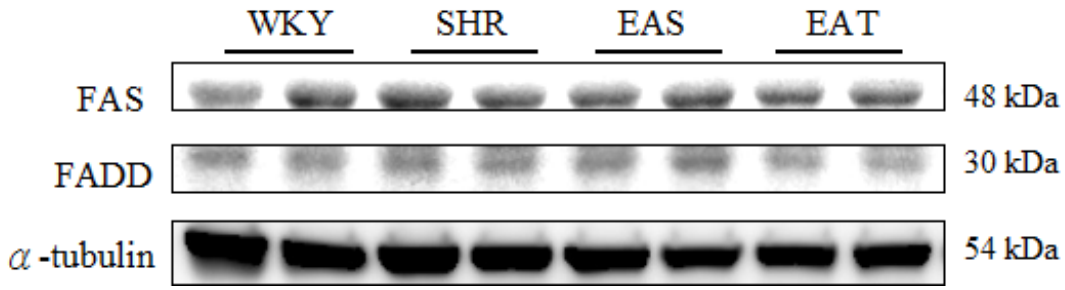


Fig 3-5. Protein levels of the cardiac hypertrophy markers.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong) for 3 weeks and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 10-12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA).Compared with WKY, *P<0.05, **P<0.01, ***P<0.001. Compared with SHR, #P<0.05, ##P<0.01. Compared with EAS, △P<0.05, △△P<0.01, △△△P<0.001.

Fig 3-6

A.



B.

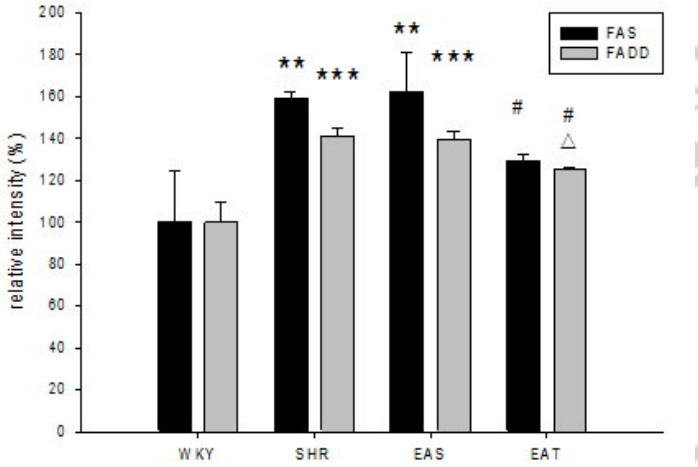
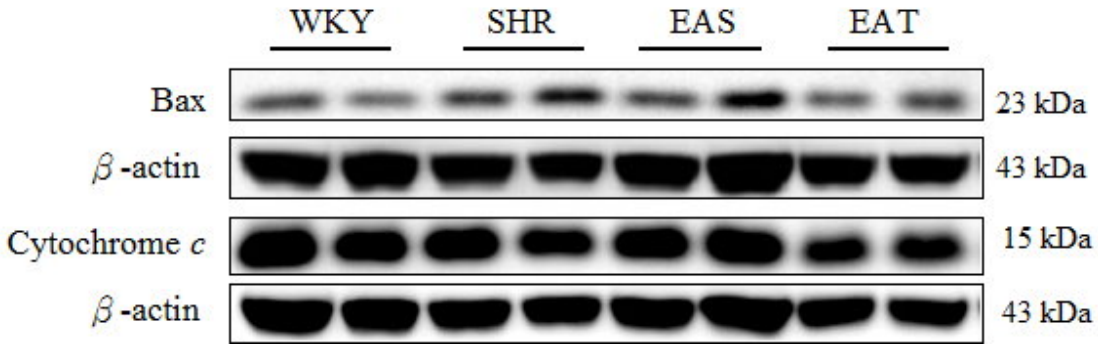


Fig 3-6. The protein expression levels of FAD and FADD extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong) for 3 weeks and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA).Compared with WKY, **P<0.01, ***P<0.001. Compared with SHR, #P<0.05. Compared with EAS, △P<0.05.

Fig 3-7

A.



B.

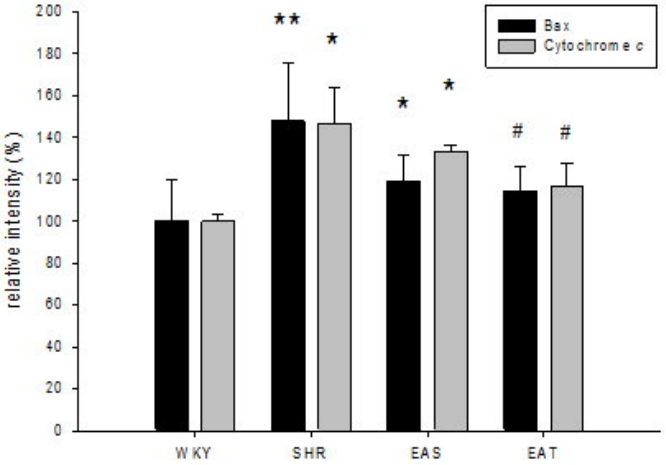
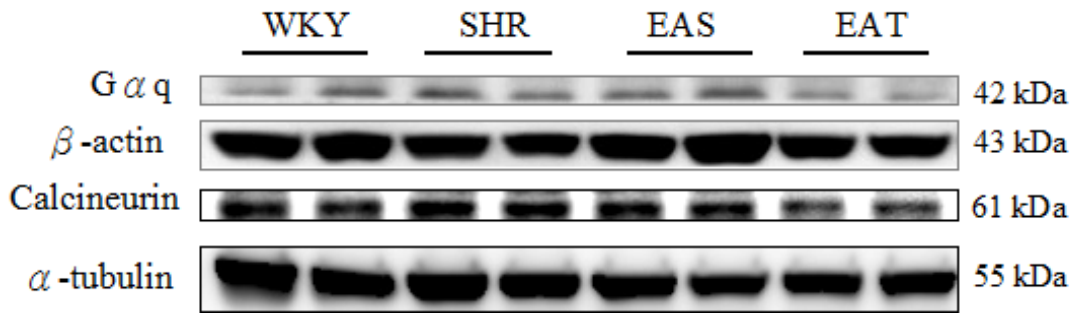


Fig 3-7. The protein expression levels of Bax and Cytochrome c extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong) for 3 weeks and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA).Compared with WKY, *P<0.05, **P<0.01. Compared with SHR, #P<0.05.

Fig 3-8

A.



B.

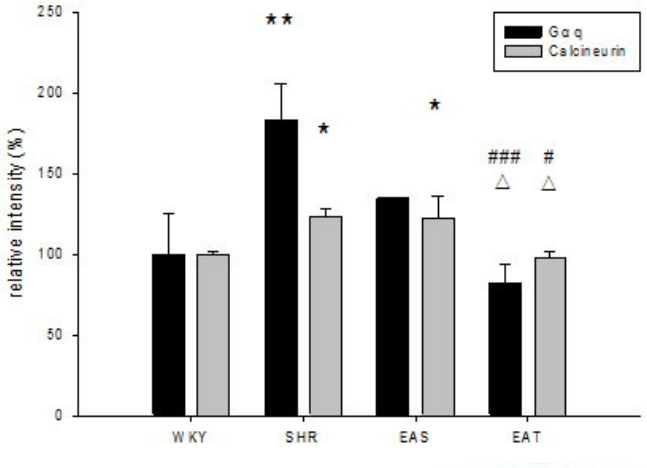


Fig 3-8. The protein expression levels of Gαq and Calcineurin extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong) for 3 weeks and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA).Compared with WKY, *P<0.05, **P<0.01. Compared with SHR, #P<0.05, ###P<0.001. Compared with EAS, △P<0.05.

Fig 7-1.

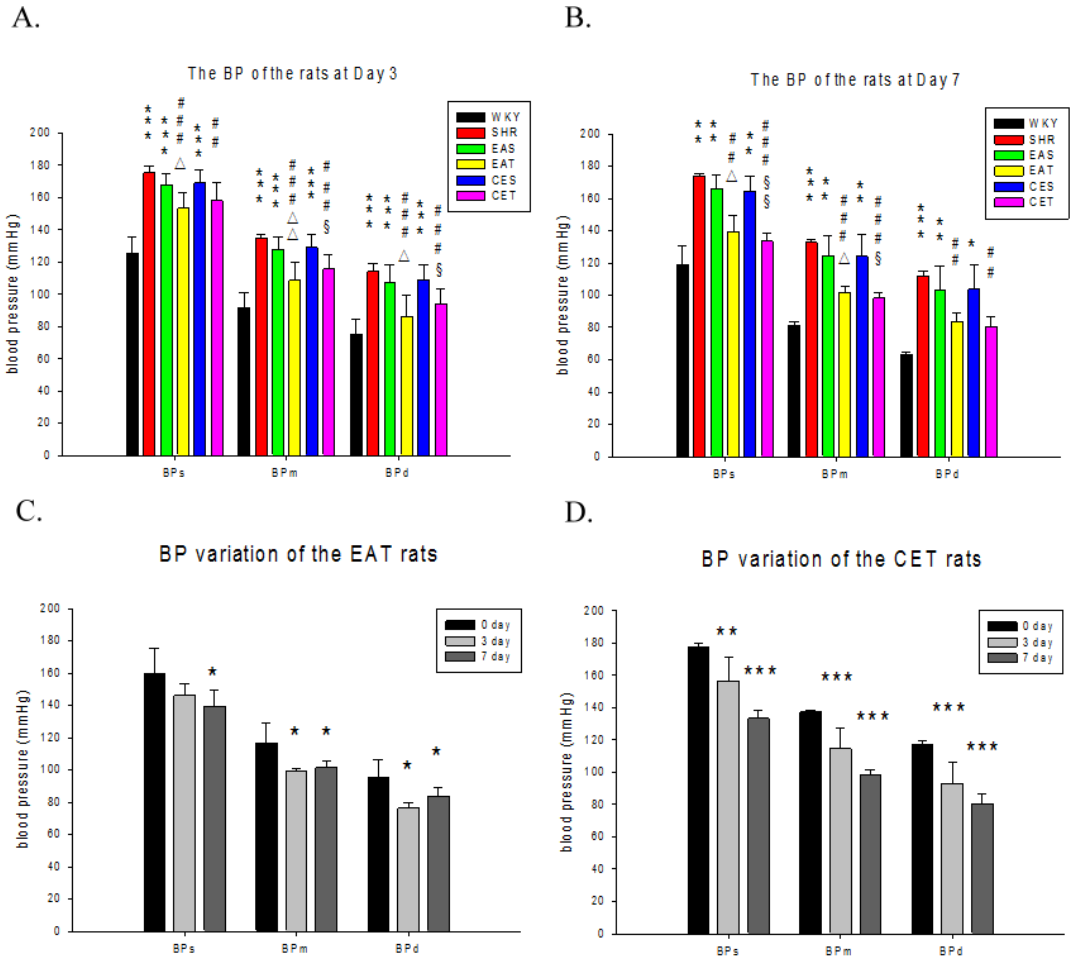
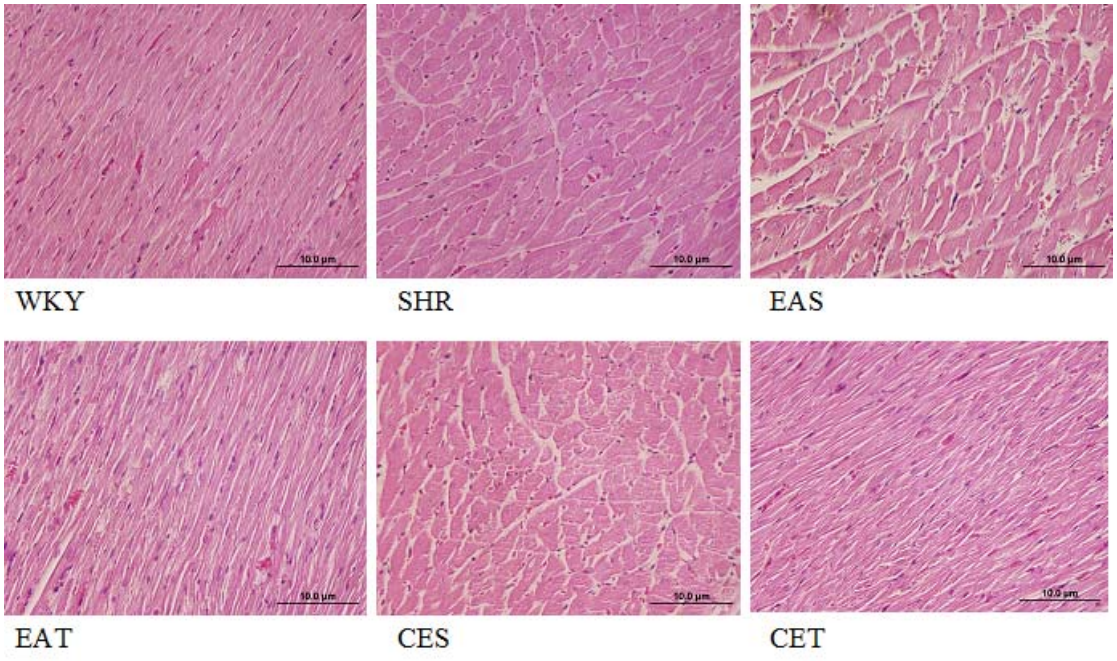


Fig 7-1. The blood pressures of the rats for treated a week

A) The charts of the third day blood pressure for rats B) The charts of the seventh day blood pressure for rats C) BP variation of the EAT rats in this 1-week treatment. D) BP variation of the CET rats in this 1-week treatment. Data are expressed as mean±SD. (Compared with WKY, **P<0.01, ***P<0.001. Compared with SHR, #P<0.05, ##P<0.01, ###P<0.001. Compared with EAS, △P<0.05,△△P<0.01, in Fig 9A and 9B. Compared with 0 day, *P<0.05, **P<0.01, in Fig 9C and 9D.)

Fig 7-2

A.



B.

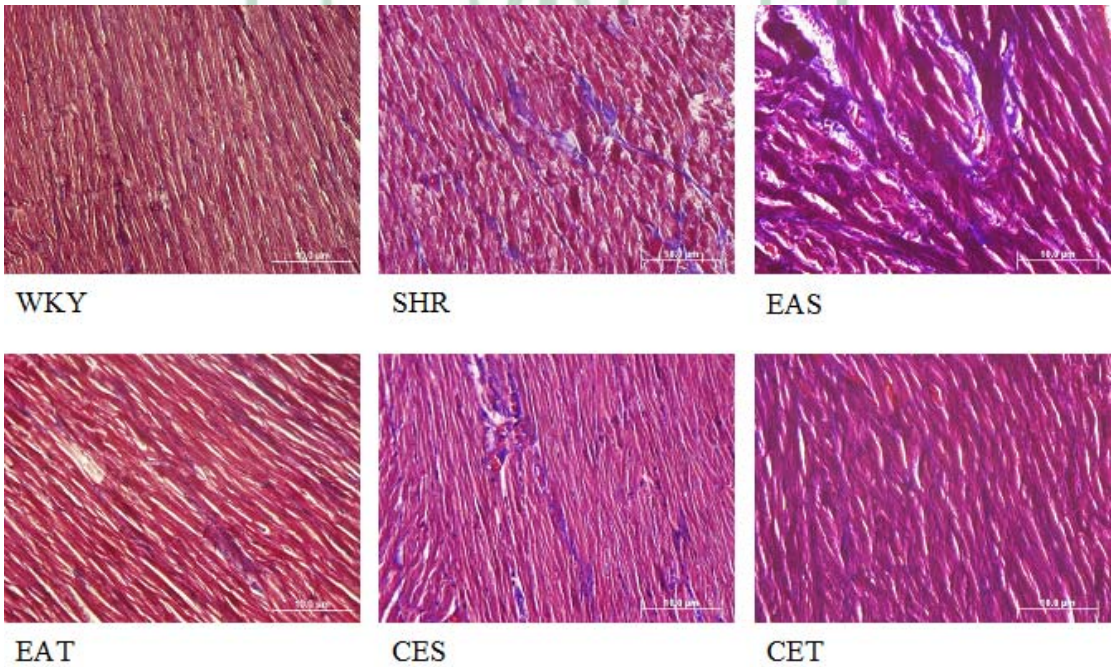


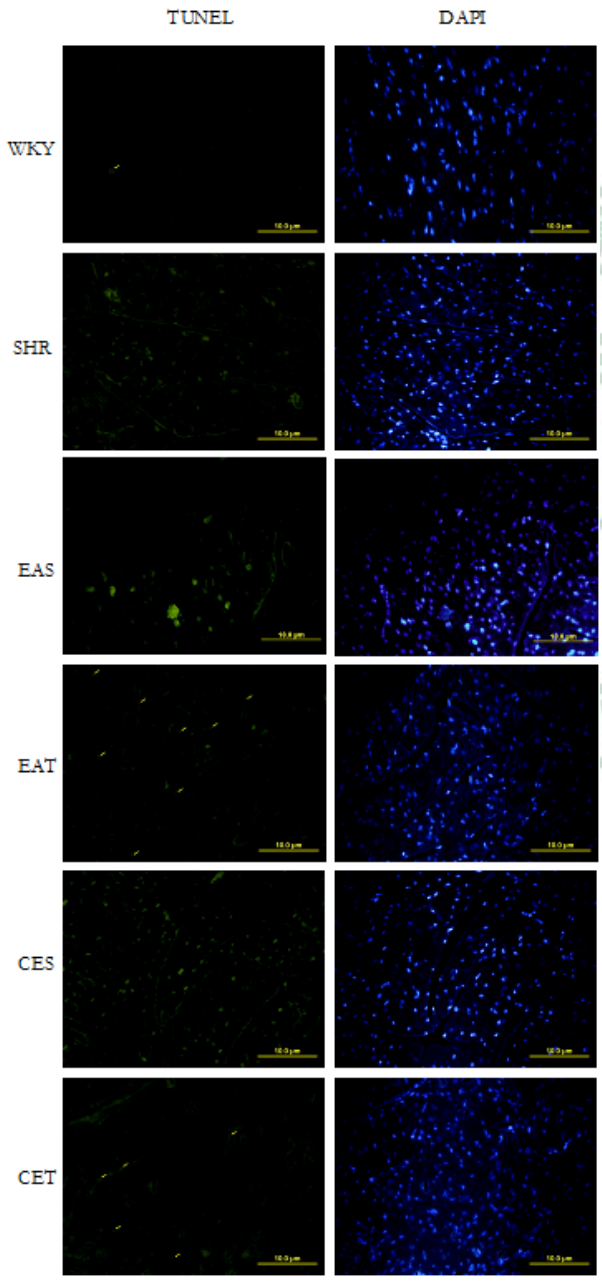
Fig 7-2. Transverse sections of LV cardiomyocytes of the rats.

A) H&E staining (400X). The cardiomyocytes of SHR, EAS and CES rats were larger and more disarrayed than WKY. After one week of treatment in Taichong, the size and arrangement of EAT and CET cardiomyocytes were

reversed. B) Masson's trichrome staining (400X). The cardiomyocytes of SHR showed more blue stain of collagen aggregation (the arrows) than WKY, and so did the EAS and CES. After one week of treatment in Taichong, the stain was almost disappeared in the LV sections of EAT and CET.

Fig 7-3

A.



B.

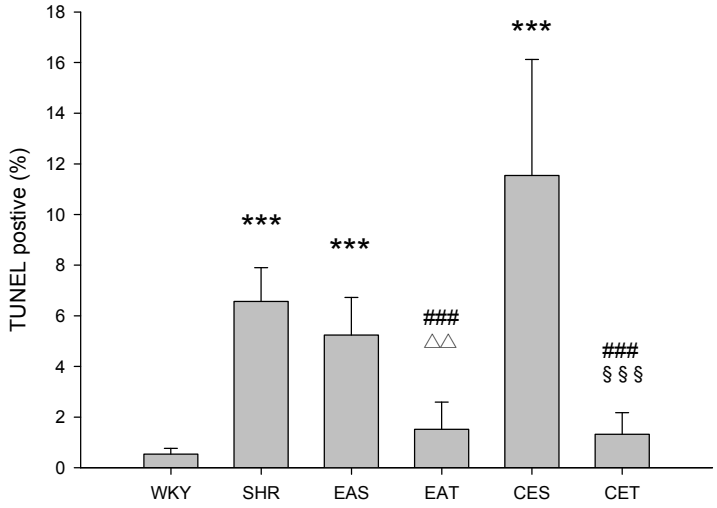
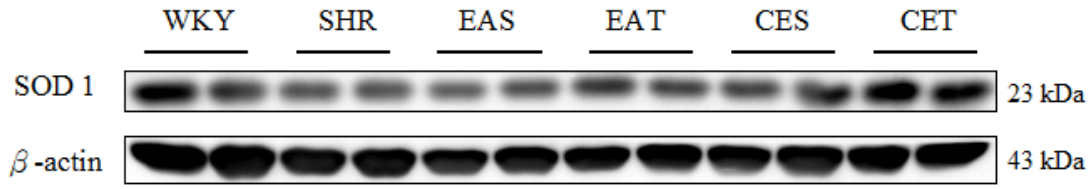


Fig 7-3. TUNEL assay of LV sections of the rats.

More TUNEL spots were detected in LV of SHR, EAS and CES rats than WKY, while the LV section of EAT showed less TUNEL spots than SHR and EAS. B) We made the figure according the result has shown that the apoptosis situation was significant decreased. Compared with WKY, ***P<0.001. Compared with SHR, ###P<0.001. Compared with EAS, △△P<0.01. Compared with CES, §§§P<0.001.

Fig 7-4

A.



B.

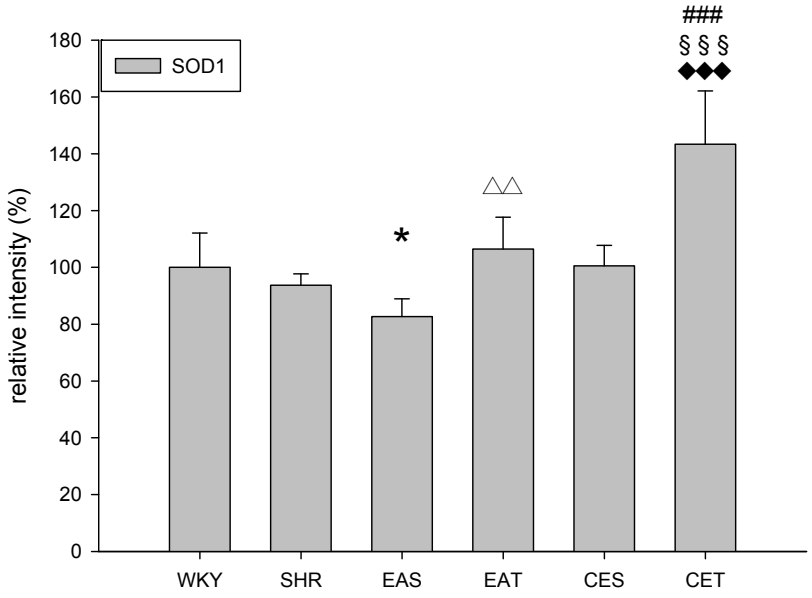
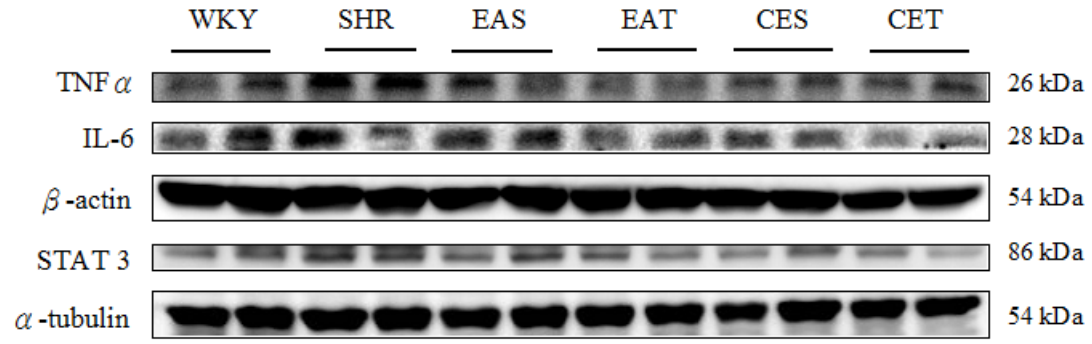


Fig 7-4. The protein expression levels of SOD 1 extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 1 week and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). Compared with WKY, *P<0.05. Compared with SHR, ###P<0.001. Compared with EAS, △△P<0.01. Compared with CES, §§§P<0.001.

Fig 7-5

A.



B.

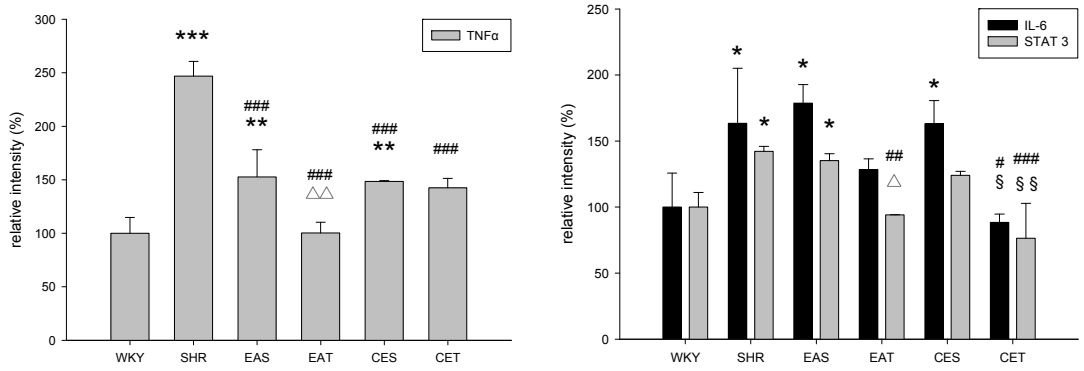
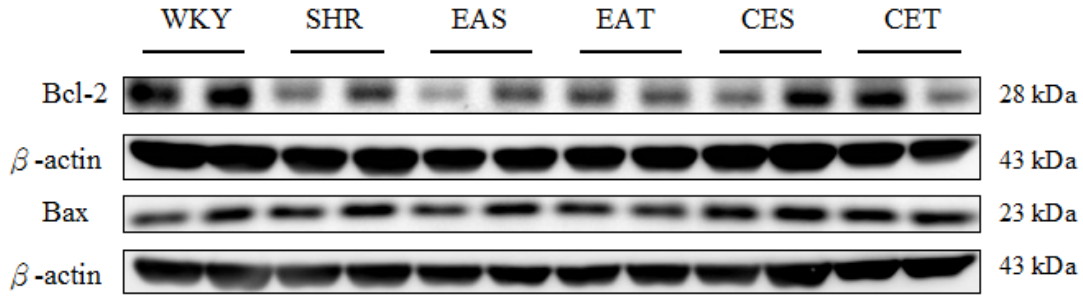


Fig 7-5. The protein expression levels of TNFα, IL-6 and STAT 3 extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 1 week and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 10-12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). Compared with WKY, *P<0.05, **P<0.01, ***P<0.001. Compared with SHR, #P<0.05, ##P<0.01, ###P<0.001. Compared with EAS, △P<0.05, △△P<0.01. Compared with CES, §§P<0.01, §§§P<0.001.

Fig 7-6

A.



B.

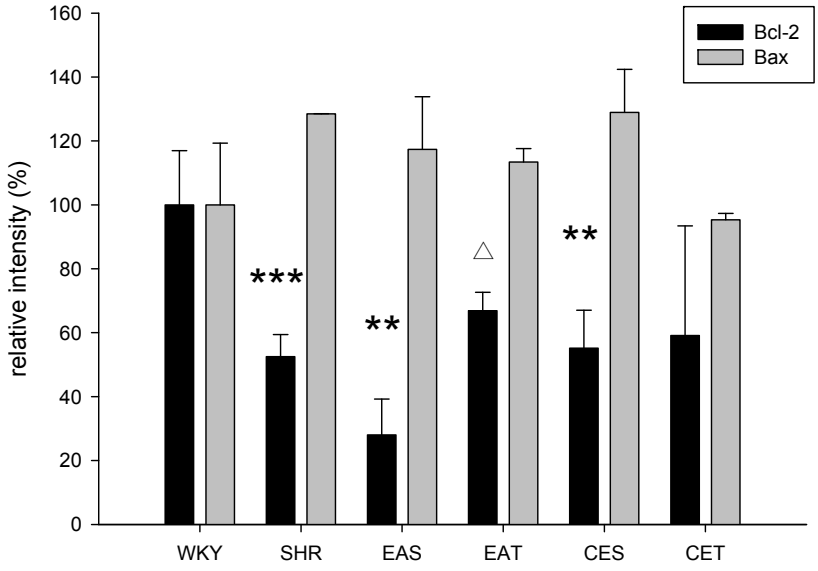
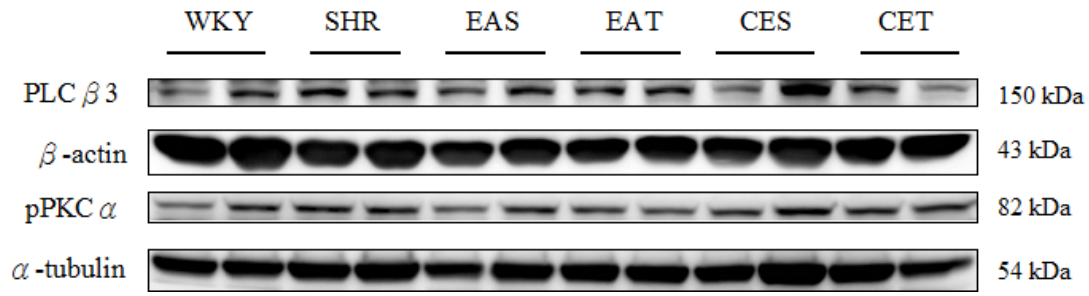


Fig 7-6. The protein expression levels of Bcl-2 and Bax extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 1 week and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). Compared with WKY, **P<0.01, ***P<0.001. Compared with EAS, △P<0.05.

Fig 7-7

A.



B.

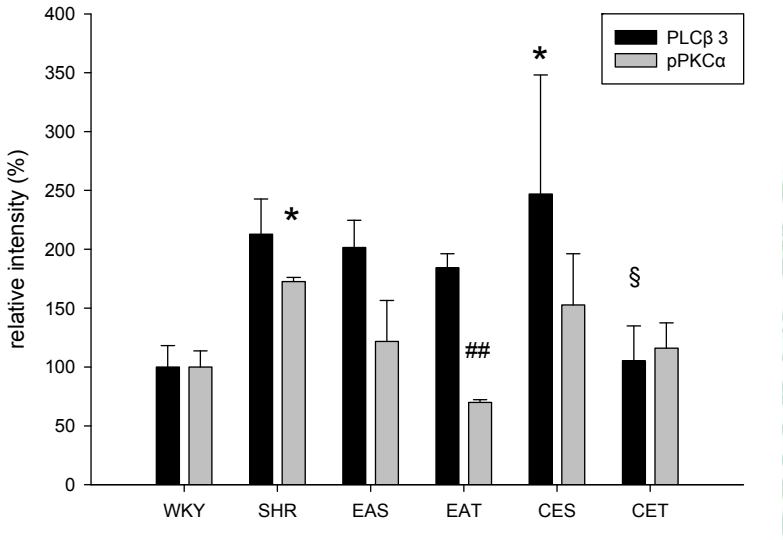
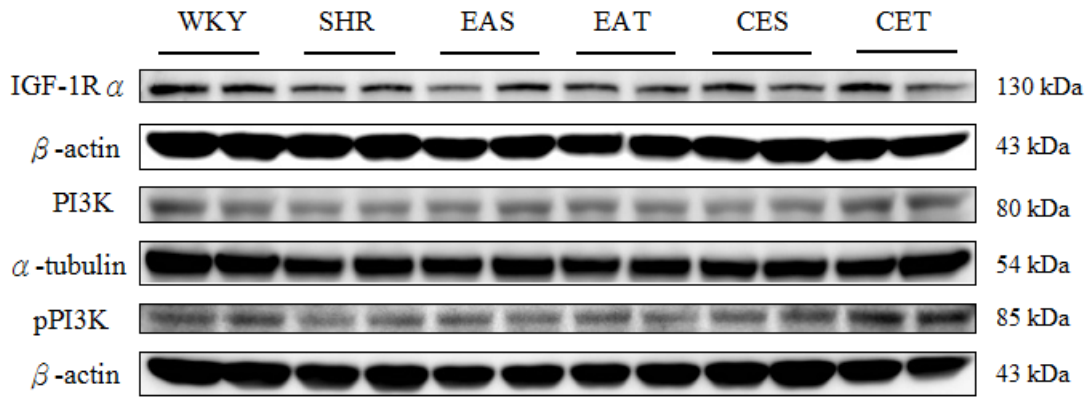


Fig 7-7. The protein expression levels of PLCβ3 and pPKCα extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 1 week and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 10 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). Compared with WKY, *P<0.05. Compared with SHR, ##P<0.01. Compared with CES, §P<0.05.

Fig 7-8

A.



B.

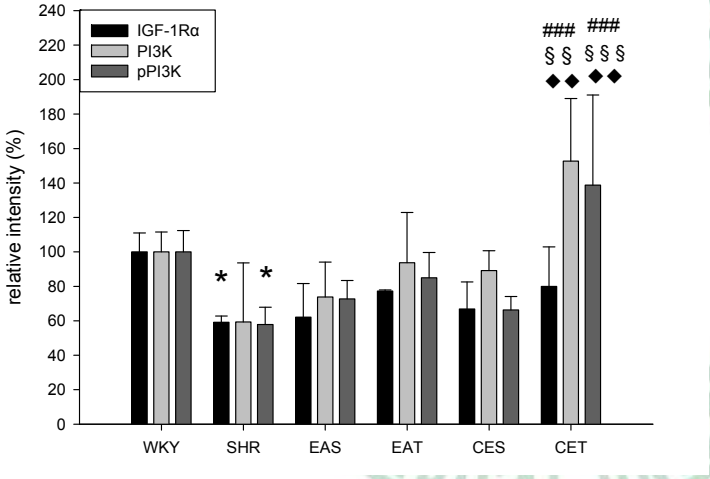
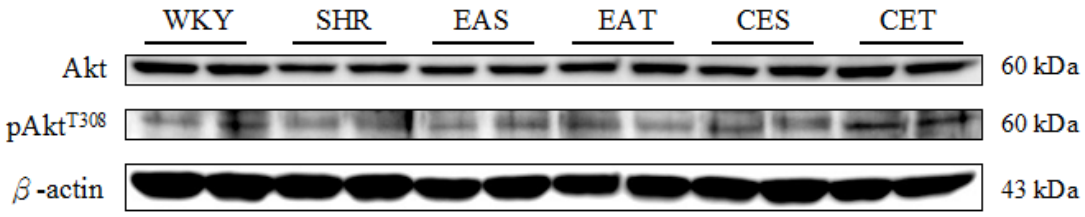


Fig 7-8. The protein expression levels of IGF-1Rα, PI3K and pPI3K extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 1 week and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 10 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). Compared with WKY, *P<0.05. Compared with SHR, ###P<0.001. Compared with CES, §§P<0.01, §§§P<0.001. Compared with EAT, ◆◆P<0.01.

Fig 7-9

A.



B.

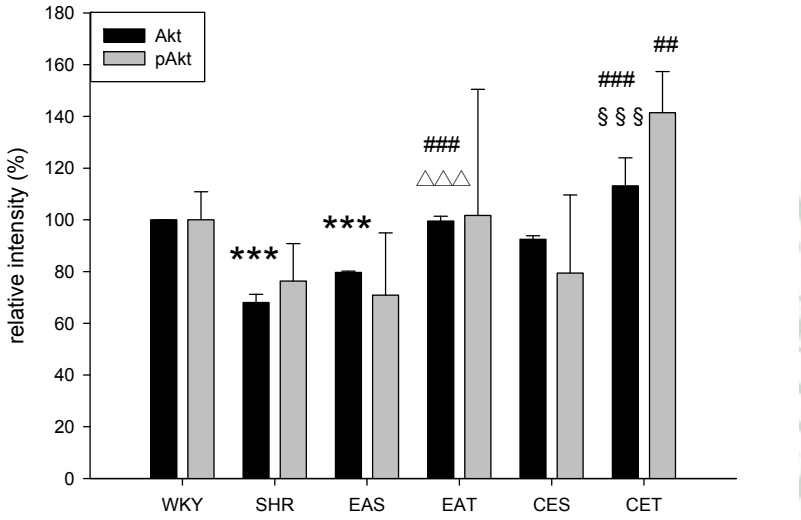


Fig 7-9. The protein expression levels of Akt and pAkt extracted from heart tissue were quantified by Western blotting analysis.

The rats of all the groups were treated (WKY, the normal control; SHR, the hypertensive control; EAS, electro-acupuncture on sham-point; EAT, electro-acupuncture on Taichong; CES, catgut-embedding on sham-point; CET, catgut-embedding on Taichong) for 1 week and then the hearts were harvested and lysed. (A) Total protein of cell extracts was separated by 12 % SDS-PAGE, and transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). Compared with WKY, ***P<0.001. Compared with SHR, ##P<0.01, ###P<0.001. Compared with EAS, △△△P<0.001. Compared with CES, §§§P<0.001.

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