ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Oct; 20 (10): 923 - 928
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Effects of perindopril, propranolol, and dihydrochlorothiazide on cardiovascular remodelling in spontaneously hypertensive rats[1]

SU Jin-Zi[2], CHEN Song-Cang[3], WU Ke-Gui, CHEN Da-Guang, RUI Hong-Bing[4], WANG Xiang-Yu, WANG Hua-Jun (*Department of Cardiology, First Affiliated Hospital of Fujian Medical University, Fujian Institute of Hypertension Research, Fuzhou 350005, China*)

KEY WORDS hypertension; left ventricular hypertrophy; propranolol; angiotensin-converting enzyme inhibitors; thiazide diuretics; inbred SHR rats

ABSTRACT

AIM: To investigate the effects of perindopril, propranolol, and dihydrochlorothiazide on artery wall thickening, left ventricular hypertrophy, and cardiac fibrosis in spontaneously hypertensive rats (SHR). **METHODS**: After measurement of systolic blood pressure (SBP), 16-wk-old ♂ SHR were randomly divided into 3 groups (each $n = 10$), given perindopril (Per, 5 mg·kg^{-1}·d^{-1}), propranolol (Pro, 40 mg·kg^{-1}·d^{-1}), dihydrochlorothiazide (DCT, 100 mg·kg^{-1}·d^{-1}) respectively by gavage for 12 wk. Sex-, age-, and number-matched untreated SHR and normotensive Wistar Kyoto rats (WKY) served as controls. When the treatment finished, body weights (BW) and SBP were measured before decapitation of the rats. The heart was excised rapidly, the left ventricle was weighed and then subjected to collagen content analysis. Vascular wall and lumen ratio from aorta, renal arteries and branch Ⅲ vessels of mesenteric arteries were determined morphometrically. **RESULTS**: Treated rats in 3 groups showed a lower SBP and the ratio of left ventricle weight to body weight (LVW/BW) compared with WKY. Artery wall thickening was similarly inhibited in the treated groups. Per and Pro inhibited cardiac fibrosis, but collagen concentration increased in DCT treated SHR [collagen volume fraction (CVF): 19 ± 4 *vs* SHR 14 ± 4, $P < 0.05$; perivascular collagen fraction (PVCF): 84 ± 7 *vs* SHR 79 ± 5, $P < 0.05$]. **CONCLUSION**: Per and Pro inhibited, but DCT promoted, cardiac fibrosis.

INTRODUCTION

Essential hypertension is always accompanied with artery wall thickening, left ventricular hypertrophy (LVH) and cardiac fibrosis. These structural changes in heart and blood vessels are known as cardiovascular remodelling. Both small and large arteries are implicated in the mechanisms of hypertension. Vascular remodelling accounts for the hypertensive target organs damage, and the heart structural alterations are responsible for the increasing morbidity and mortality of cardiac events. Therefore, treatment of hypertension shall aim not only at the optimal control of blood pressure, but also the prevention of the cardiovascular remodelling[1]. Although many antihypertensive drugs, such as angiotensin converting enzyme inhibitor (ACEI), β-adrenoceptor blocker, diuretic, were effective in reducing blood pressure, they affected cardiovascular remodelling by different pharmacological actions, and usually in a dose-dependent way[2]. So far, ACEI was the most effective in the reversion of LVH, prohibition of vascular remodelling and target organ damages[3]. β-Adrenoceptor blocker was of minor efficacy[3]. The results from diuretic had been contradictory[4]. Recently, large scale clinical trials[5] together with animal experiments revealed the advantages and disadvantages of many antihypertensive drugs. However, influence of different kinds of antihypertensive drugs on several important aspects,

[1] Project supported by the National Natural Science Foundation of China, № 39270329.
[2] Correspondence to Dr SU Jin-Zi. Phn 86-591-335-7199, ext 756 or 324. Fax 86-591-331-8716. E-mail sujinzi@163.net
[3] Now in *Metabolism Research Unit, University of California, San Francisco CA* 94143, *USA*.
[4] Now in *Department of Hematology, First Affiliated Hospital of Fujian Medical University, Fuzhou* 350005, *China*.
Received 1998-12-21 Accepted 1999-04-05

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Oct; 20 (10)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

such as cardiac fibrosis, was not well documented. Therefore, comparison of such influence among these drugs was necessary to be carried out to outline their favorable and unfavorable characteristics on the basis of therapeutic purpose. This study investigated the effects of 3 antihypertensive drugs: perindopril (Per, an ACEI), propranolol (Pro, a beta-adrenoceptor blocker), and dihydrochlorothiazide (DCT, a diuretic), with a comparably marked antihypertension dose, on the vascular wall thickening from elastic conducting vessels, large muscle artery and anteriolar resistant arteries, LVH and cardiac fibrosis in SHR.

MATERIALS AND METHODS

Drugs and chemicals Per was the product of France Service International BV. Pro and DCT were qualified products from Tianjin Lisheng Pharmaceutical Factory and Changzhou No 4 Pharmaceutical Factory respectively. Hematoxylin-eosin and picrosirius red were from Sigma (USA). Other chemicals were of either AR or molecular biology grade.

Rats and treatment Rats were offsprings of breeders derived from Shanghai Institute of Hypertension. After measurement of systolic blood pressure (SBP), 16-wk-old SHR (Body weight: 178 g ± *s* 14 g) were randomly divided into 3 groups (each n = 10), each group received Per 5 $mg \cdot kg^{-1} \cdot d^{-1}$, Pro 40 $mg \cdot kg^{-1} \cdot d^{-1}$, DCT 100 $mg \cdot kg^{-1} \cdot d^{-1}$ respectively for 12 wk. Drugs were mixed with small amount of milk powder and water and were given by gavage. Only milk powder was given to sex-, age-, and number-matched untreated SHR and normotensive WKY controls. Measurements were performed when the treatment was finished.

Systolic blood pressure (SBP) and left ventricular weight (LVW) to body weight (BW) ratio measurement SBP was measured using tail-cuff technique (MRB-Ⅲ A computer control sphygmomanometer for rats, Shanghai Institute of Hypertension) before and at the end of the treatment. Rats were weighed before decapitation, the heart excised, left ventricles including interventricular septum were weighed. LVH was assessed by LVW/BW.

Determination of cardiac fibrosis Tissues from left ventricles were fixed in 10 % formaldehyde, paraffin enbedded. Sections (6 μm) were processed using Picrosirius Red method[6]. Results were analyzed under microscope and with video image planimeter (VP32, Australia).

Morphometric analysis of vascular wall thickening[7] The abdominal aorta of the rats was incised, perfused with sodium nitroprusside and heperin for 5 min to assure maximal vasodilation and anti-coagulation. Thereafter, 2 % formaldehyde in Hanks' balance solution was used under a pressure of 294 Pa for fixation of vessels. abdominal aorta (elastic conducting vessels), renal artery (large muscle artery), and mesenteric artery branch Ⅲ (anteriolar resistant arteries) were chosen for sections. Vessels were processed and embedded in paraffin for transverse sections. Sections (6 μm) were stained with hematoxylin-eosin. Vascular wall and lumen ratio was determined by video image planimeter (VP32, Australia).

Statistical analysis Data were expressed as $\bar{x}$ ± s. ANOVA with Newman-Kuels procedure was used to evaluate the differences among treated SHR, untreated SHR and WKY rats.

RESULTS

SBP was reduced by 3 drugs in SHR SBP in 3 treated groups and SHR control were equal before the treatment began. During the 12 wk of treatment, SBP in untreated SHR increased (3.1 ± 2.4) kPa (P < 0.05) compared with that before treatment, while SBP in WKY stayed at a nearly stable level. Each drug effectively decreased the SBP in SHR rats, similar to that of WKY rats (Tab 1).

Inhibitory effects on LVH The ratio of LVW to BW in adult untreated SHR rats was much higher than that of WKY. Per, Pro, and DCT decreased the value of LVW/BW in SHR. No difference was showed in the inhibitory effects among the drugs. The index did not attain a complete normalization compared with WKY rats (Tab 1).

Inhibition of vascular wall thickening in SHR An obvious artery wall thickening was seen in abdominal aorta, renal artery, and mesenteric artery branch Ⅲ vessels in adult SHR. The lumen area of the arteries decreased, resulting in an increase in the

Tab 1. Effects of perindopril (5 mg·kg^{-1}·d^{-1}), propranolol (40 mg·kg^{-1}·d^{-1}), dihydrochlorothiazide (100 mg·kg^{-1}·d^{-1}) on systolic blood pressure and left ventricular hypertrophy in SHR. $n = 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* SHR. $^dP > 0.05$, $^eP < 0.05$ *vs* WKY. $^hP < 0.05$ *vs* SBP before treatment in untreated SHR.

Groups	Systolic blood pressure/kPa		Decrease in systolic blood pressure/kPa	Left ventricular weight to body weight, mg·g^{-1}
	Before	After		
WKY	18.3 ± 1.6^b	18.4 ± 1.6^b	-0.1 ± 0.4^b	2.8 ± 0.6^b
SHR	26.1 ± 2.8	29.1 ± 2.7^h	-3.1 ± 2.4	4.8 ± 0.9
SHR_{Per}	25.0 ± 1.2	18.6 ± 1.3^{bd}	6.3 ± 1.3^{be}	3.5 ± 0.5^{be}
SHR_{Pro}	27.0 ± 2.6	20.2 ± 2.4^{bd}	6.8 ± 1.9^{be}	3.8 ± 0.8^{be}
SHR_{DCT}	26.0 ± 2.1	19.8 ± 1.5^{bd}	6.1 ± 2.6^{be}	3.6 ± 0.6^{be}

ratio of wall to lumen ratio. Histological analysis found the number of smooth muscle cell layers and smooth muscle cell density in arteries of SHR were more than that in WKY rats. Treatment with the drugs similarly prevented the occurrence of vascular remodelling in SHR (Tab 2).

Effects on cardiac fibrosis In left ventricles of SHR, cardiac fibrosis was evident by the collagen accumulation in the interstitium and around the intramyocardial coronary artery. Collagen volume fraction reached 14.29 % in SHR. Both Per and Pro prevented cardiac fibrosis and necrosis of cardiomyocytes in SHR. In DCT-treated rats, the CVF and PVCF were even higher than those of untreated SHR. Necrosis of cardiomyocytes and reparative fibrosis were also observed in this group as well as SHR rats (Tab 2, Fig 1).

DISCUSSION

Present study investigated the therapeutic aspects of three kinds of the most commonly used antihypertensive drugs perindopril, propranolol, and dihydrochlorothiazide on hereditary hypertension. It is found that, with the given dosages, they were similarly effective in lowering blood pressure, prohibiting the vascular remodelling and left ventricular hypertrophy (LVH) in term of LVW/BW, regardless of the different mechanisms of the drugs. In most hypertensive animal models, left ventricle mass can be reduced when blood pressure decreased. However, high risk of cardiac events in hypertensive patients is determined not only by LVH, but cardiac fibrosis as well[8]. The differential effects of antihypertensive drugs on cardiac fibrosis attracted a lot of interest from clinical practice. Data from this study showed perindopril and propranolol were effective to prohibit cardiac fibrosis in SHR rats. This, together with other reports[4], demonstrated that ACEI and β-adrenocepter blocker were qualified in reducing the raised risk resulted from cardiovascular remodelling. The supression of renin-angiotensin system and sympathetic activity would mainly contribute to the results. Diuretics were among the most commonly used hypotensive drugs. Small dose of

Tab 2. Effects of perindopril, propranolol, and dihyfrochlorothiazide on the artery wall thickening and cardiac fibrosis in SHR rats. $n = 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* SHR. $^eP < 0.05$ *vs* WKY. $^hP < 0.05$ *vs* SHR_{DCT}.

Group	Vascular wall to lumen ratio			Collagen fraction/%	
	Abdominal aorta	Mesenteric artery branch Ⅲ	Renal artery	Heart	Perivascular
WKY	0.23 ± 0.07^b	0.33 ± 0.06^b	0.31 ± 0.05^b	6.0 ± 1.8^{bh}	57 ± 12^{bh}
SHR	0.39 ± 0.06	0.59 ± 0.13	0.46 ± 0.06	14 ± 4^h	79 ± 5^h
SHR_{Per}	0.23 ± 0.05^b	0.34 ± 0.05^b	0.31 ± 0.04^b	7.3 ± 1.4^{beh}	61 ± 13^{beh}
SHR_{Pro}	0.25 ± 0.06^b	0.34 ± 0.05^b	0.32 ± 0.04^b	7.5 ± 1.5^{beh}	59 ± 9^{beh}
SHR_{DCT}	0.27 ± 0.05^b	0.35 ± 0.05^b	0.32 ± 0.03^b	19 ± 4^{be}	84 ± 7^{be}

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Oct; 20 (10)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

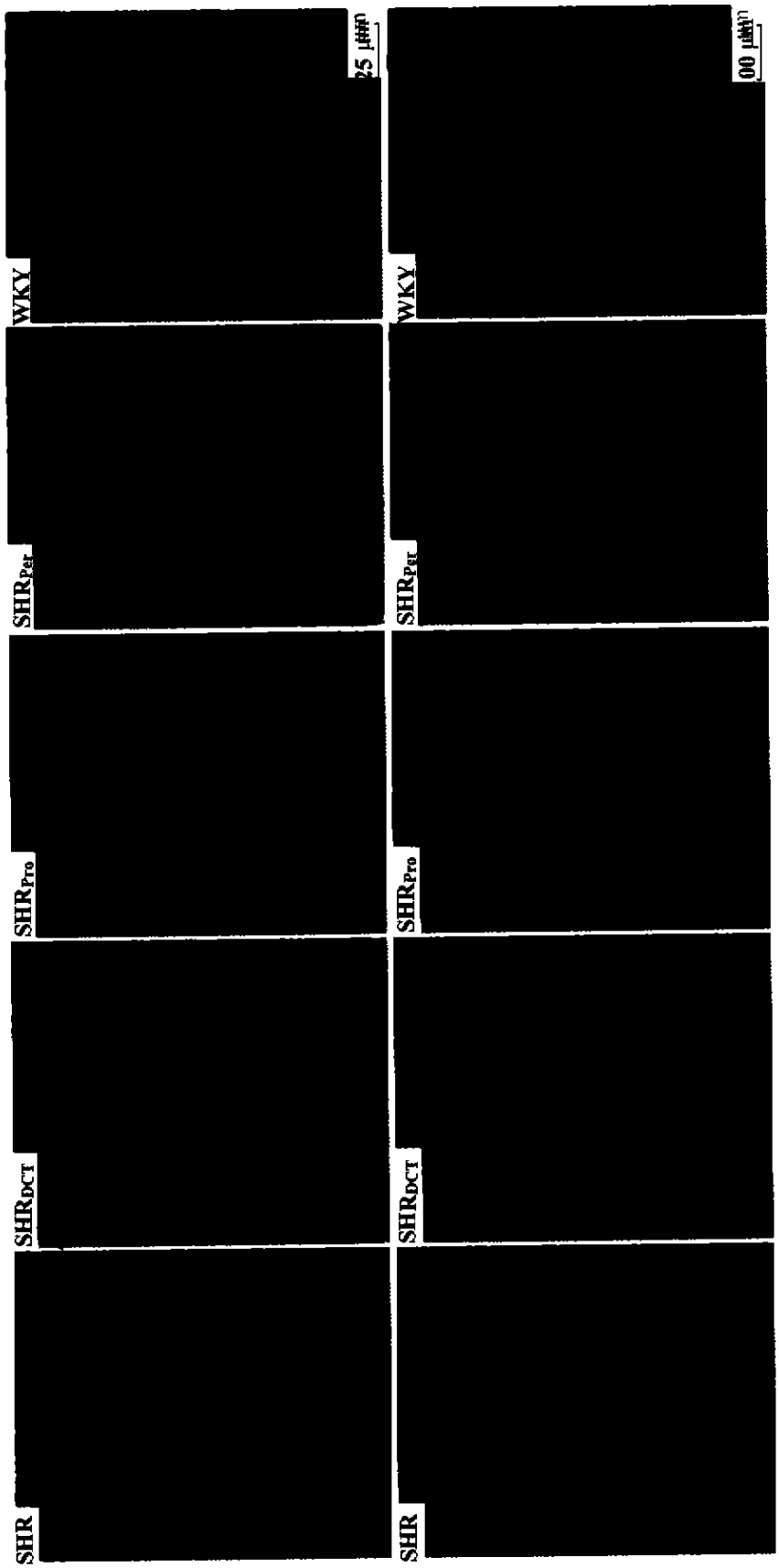

Fig 1. Morphological and morphometric accessment of cardiac fibrosis of left ventricles in the treated and control group, showing the collagen volume fraction (upper, ×400) and perivascular collagen fraction (lower, ×100). Stained with picrosirius red method.

thiazides diuretic was sufficient to reduce blood pressure, but less effective in regressing LVH[4]. Moderate to high dose of thiazides therapy was associated with an increasing risk of cardiac events[9]. In this study, dihydrochlorothiazide acerbated the cardiac fibrosis in SHR, indicated that the mechanism of cardiac fibrosis was different from that of cardiomyocytes hypertrophy. These results may well interpret that high dose of thiazide diuretic treatment raised the incidence of cardiac arrest in hypertensive patients and the heart-toxicity. Although some investigator hold that the disturbing effects of diuretic on lipid metabolism may be little clinically significant[10], it was believed that increasing risk resulted from the disturbing effects of high dose of thiazide diuretic on lipid metabolism, causing hypokalemia and a significant increase in plasma renin concentration[11]. Although DCT normalized the blood pressure, it actually promoted cardiac fibrosis possibly by worsening the metabolic and hormonal disturbance. Therefore, thiazides diuretics were unfavorable for reducing of high risk of cardiac events, suggesting that DCT was less safe when other diuretic such as imdapamide had been available[12]. Further study shall be focused on the relationship of dosage-effect of thiazide diuretic in the promotion of fibrosis to assure the safety of clinical application, and the exact mechanism of DCT on the fibrotic pathogenesis must be further investigated.

Arteries wall thickening, which helped maintain elevated blood pressure and caused target organs damages, was another critical factor for mortality in hypertension. Thus, ameliorating vascular remodelling is among the therapeutic goats. Antihypertensive treatment had been proven to be effective in prohibiting arteries wall thickening and the resulting target organ damages[13]. Different antihypertensive drugs, though effective in reducing blood pressure, affected hormonal factors in diverse ways. Our data showed the three drugs effectively regressed the remodelling in elastic conducting vessels, large muscle artery and anteriolar resistant artery. Similar reports[14] with ours demonstrated that artery wall thickening was resulting from a rising arterial pressure rather than hormonal factors, and that the mechanism of arteries wall thickening was different from that of cardiac fibrosis.

In conclusion, present study showed that vascular remodelling and LVH in SHR regressed with the reduction of blood pressure. ACEI and β-blocker were effective in reducing of artery wall thickening, left ventricular hypertrophy and cardiac fibrosis. Although dihydrochlorothiazide was effective in antihypertension and decreasing arteries wall thickening, it promoted the cardiac fibrosis at a large dosage. These provide a clinical implication of drug choice in the protection of target organ damage in hypertensive patients.

REFERENCES

1 Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991; 83: 1849 - 65.
2 Black MJ, Kanellakis P, Bobik A. Role of angiotensin II in early cardiovascular growth and vascular amplifier development in spontaneously hypertensive rats. J Hypertens 1997; 15: 945 - 54.
3 Schmieder RE, Martus P, Klingbeil A. Reversal of left ventricular hypertrophy in essential hypertension. A meta-analysis of randomized double-blind studies. JAMA 1996; 275: 1507 - 13.
4 Siscovick DS, Raghunathan TE, Psaty BM, Koepsell TD, Wicklund KG, Lin X, *et al*. Diuretic therapy for hypertension and the risk of primary cardiac arrest. N Engl J Med 1994; 330: 1852 - 7.
5 Senior R, Imbs JL, Bory M, Amabile G, Denis B, Zannad F, *et al*. Indapamide reduces hypertensive left ventricular hypertrophy: an international multicenter study. J Cardiovasc Pharmacol 1993; 22 Suppl 6: S106 - 10.
6 Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy: a specific method for collagen detection in tissue sections. Histochem J 1979; 11: 447 - 55.
7 Chen SC, Chen DG, Jin XQ, Hu WY, Wang HJ. Mechanism to prevent genetic hypertension by early captopril treatment in spontaneously hypertensive rats. Chin J Pharmacol Toxicol 1995; 9: 175 - 9.
8 McLenachan JM, Dargie HJ. A review of rhythm disorders in cardiac hypertrophy. Am J Cardiol 1990; 65: 42G - 44G.
9 Contard F, Glukhova M, Marotte F, Narcisse G, Schatz C, Swynghedauw B, *et al*. Diuretic effects on cardiac hypertrophy in the stroke prone spontaneously hypertensive rat. Cardiovasc Res 1993; 27: 429 - 34.
10 Moser M. Current hypertension management: separating fact from fiction. Cleve Clin J Med 1993; 60: 27 - 37.
11 Wada T, Sanada T, Ojima M, Kanagawa R, Nishikawa K, Inada Y. Combined effects of the angiotensin II antagonist candesartan cilexetil (TCV-116) and other classes of antihypertensive drugs in spontaneously hypertensive rats.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Oct; 20 (10)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Hypertens Res 1996; 19: 247 – 54.
12 Carey PA, Sheridan DJ, de Cordoue A, Guez D. Effect of indapamide on left ventricular hypertrophy in hypertension: a meta-analysis. Am J Cardiol 1996; 77: 17B – 19B.
13 Wang H, Delaney KH, Kwiecien JM, Smeda JS, Lee RM. Prevention of stroke with perindopril treatment in stroke-prone spontaneously hypertensive rats. Clin Invest Med 1997; 20: 327 – 38.
14 Karam H, Heudes D, Hess P, Gonzales MF, Loffler BM, Clozel M, *et al*. Respective role of humoral factors and blood pressure in cardiac remodeling of DOCA hypertensive rats. Cardiovasc Res 1996; 31: 287 – 95.

培哚普利、普萘洛尔与双氢氯噻嗪对自发性高血压大鼠心血管结构重塑的影响[1]

苏津自[2], 陈松苍[3], 吴可贵, 陈达光, 芮红兵[4], 王向宇, 王华军
(福建医科大学附属第一医院心血管内科, 福建省高血压研究所, 福州 350005, 中国)

目的: 探讨培哚普利、普萘洛尔与双氢氯噻嗪对SHR大鼠心血管重塑的影响. **方法**: 16周龄的雄性SHR分别接受培哚普利 (5 mg·kg^{-1}·d^{-1}), 普萘洛尔 (40 mg·kg^{-1}·d^{-1}), 与双氢氯噻嗪 (100 mg·kg^{-1}·d^{-1})治疗12周, 治疗结束后测收缩压, 左心室重与体重比. 天狼星红染色分析心肌胶原形态与含量; 形态计量法分析血管肥厚程度. **结果**: 各治疗组动物的血压治疗后均接近正常水平, 3种降压药均可逆转左心室和动脉壁肥厚, 培哚普利和普萘洛尔能防止心肌纤维化, 双氢氯噻嗪则促进心肌纤维化的进展. **结论**: 培哚普利、普萘洛尔能有效逆转心肌纤维化, 双氢氯噻嗪对心肌纤维化有不良影响.

关键词 高血压; 左心室肥厚; 普萘洛尔; 血管紧张素转化酶抑制剂; 噻嗪利尿药; 近交SHR大鼠

(责任编辑 朱倩蓉)

11th European Congress of Clinical Microbiology and Infectious Diseases

2001 Apr 1 – 4 Istanbul, TURKEY

Info: ESCMID Executive Office
c/o AKM Congress Service
P O Box
CH-4005 Basel
SWITZERLAND

Phn 41-61-686-7711. Fax 41-61-686-7788. E-mail info@akm.ch
Internet www.akm.ch/eccmid