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Effects of tetrandrine on cardiac and vascular remodeling

RAO Man-Ren[1]

Department of Cardiovascular Pharmacology, Nanjing Medical University, Nanjing 210029, China

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ABSTRACT

Tetrandrine (Tet) is an alkaloid isolated from the Chinese herb Radix of *Stephaniae tetrandrae* S Moore. Cardiac and vascular remodeling confers a very definite risk of increased cardiovascular morbidity and mortality. Remodeling reversal has been achieved in human and experimental animals treated with some antihypertensive drugs but not all. This review will focus on cardiovascular remodeling and therapeutic effects of Tet. Three models, SHR, RHR (high renin), and DOCA-Salt HR (low renin) were used. Left ventricular and vascular remodeling had been developed in rats with 8-week untreated hypertension. Tet was administrated by ig 50 $mg \cdot kg^{-1} \cdot d^{-1}$ for 9 weeks. Tet lowered SBP, left ventricular weight to body weight ratio, vascular media thickness, media to lumen ratio, cardiac and vascular wet weight, and collagen content. Tet decreased markedly the density and total number of dihydropyridine binding sites and also decreased Ca^{2+} overload in myocardium and vessels. Tet improved haemodynamic changes during remodeling especial diastolic function such as LV compliance and stiffness, increased cardiac myosin ATPase activity and Na^+-K^+, Ca^{2+} ATPase activity, and normalized vascular reactivity. Tet inhibited proliferation of vascular smooth muscle cells, induced and sensitized VSMCs to pro-apoptosis stimulation, improved the endothelial function, and increased NO production. These results suggest that Tet was not only an anti-hypertensive drug but also an excellent drug to reverse cardiac and vascular remodeling.

INTRODUCTION

Remodeling of left ventricular and large and small arteries were common complications in hypertension. Development of left ventricular remodeling serves as an adaptive phenomenon to overcome hemodynamic afterload of hypertensive vascular disease. Left ventricular and vascular hypertrophy confers a very definite risk of increased cardiovascular morbidity and mortality that is independent of the arterial blood pressure. Patients with a history of hypertension have at least a 6-fold greater risk of developing heart failure than do individuals without such a history. Heart failure in hypertension[1] can occurs as a consequence of either diastolic dysfunction or systolic abnormalities associated with detrimental chamber (LV dilatation and the development of an eccentric cardiac geometry) and interstitial (reparative fibrosis with qualitative changes in collagen) remodeling. After a myocardial infarction (MI), ventricular remodeling[2] occurs in both the myocardium surrounding the infracted tissue (border zone) and the myocardium remote from the infarct to preserve cardiac output and limit wall stress. This adaptation involves LV dilatation, changes in LV shape, myocyte hypertrophy, and interstitial myocardial fibrosis. Howerer, progressive LV remodeling after MI lead to a

[1] Correspondence to Prof RAO Man-Ren.
Phn 86-25-666-3597. E-mail Raomanren@263sina.com
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deterioration in contractile function. In hypertension, chronic vasoconstriction[3] may lead to remodeling of the contracted vessel structure in a remodeled extracellular matrix, contributing to the inward remodeling of the blood vessel as smooth muscle cells are rearranged around a smaller lumen. The resulting remodeling of small arteries may initially be adaptive, but eventually it becomes maladaptive. Many recent reports[4] have shown that angiotensin (Ang)II, the key product of the renin-angiotensin system (RAS), is one of the critical factors for myocardial hypertrophy and fibrosis as well as other stimuli, such as $[Ca^{2+}]_i$ overload, catechloamines, endothelin-1, and peptide growth factors. These changes are accepted to be the main features of cardiac and vascular remodeling. In addation either ACE inhibitors(ACEI) or Ang II type-1 receptor(AT_1) antagonists (angiotensin receptor blockers ARBs) can potently reduce these changes. Thus now the best means for preventing the risks of ventricular and vascular remodeling is to prevent its development at beginning. Cardiac and vascular hypertrophy reversal has been achieved in human and experimental animals with some anti-hypertensive agents, such as Ca^{2+} antagonists, ACEIs, ARBs, and so on. Although its precise mechanisms have not been fully elucidated.

Tetrandrine (Tet), a bis-benzylisoquinoline alkaloid isolated from the Chinese medicinal herb, Radix of *Stephania tetrandrae* S Moore, has long been used for treatment of angiona and hypertension in China[5,6]. In cardiac sarcolemma membrane vesicles Tet interacts directly with the benzothiazepine-binding site[7]. Patch-clamp studies have demonstrated that Tet inhibits both T and L calcium channel currents in ventricular and neuroblastoma cells[8,9]. Liu *et al* have indicated that Tet relaxes tail artery helical stripe (as a model of resisance vessel) stimulated by KCl and inhibits KCl-induced intracellular Ca^{2+} increase and L-type valtage-dependent Ca^{2+} channel currents[10]. Furthermore Tet inhibits thapsigargin-induced intracellular Ca^{2+} increase suggesting that Tet also may interfere with the interaction between thapsigargin and Ca^{2+} adenosine triphosphatase.

Ca^{2+} plays a pivotal role in hypertension and as a second messenger in many cells. Some reports suggests that calcium antagonists may cause reversal of cardiac and vascular hypertrophy and regression of left ventricular hypertrophy[11] is due to their direct effects on the total number of dihydropyridine binding sites, intracellular Ca^{2+}, and collagen. However, it is yet unknown whether Tet as an anti-hypertensive agent or calcium antagonist or not influences cardiac and vascular remodeling. Many investigations have done in my lab over ten years. This review will focus on effects of Tet on cardiac and vascular remodeling after hypertension for a long time, while hypertrophy have developed on heart and vessel.

EFFECTS OF TET ON CARDIAC REMODELING IN HYPERTENSION

Three experimental models of hypertension Spontaneously hypertensive rats (SHR)[12], renovascular hypertensive rats (RHR[13], two kidney one clip Goldblatt hypertension, high renin), DOCA-salt hypertension rats (DOCA-salt HR, low renin) were used. Eight weeks were allowed to induce and stabilize cardiac hypertrophy after hypertension have developed[14]. Tet was given 50 $mg \cdot kg^{-1} \cdot d^{-1}$ by gastric tube for 9 weeks (Tab 1).

Effects of Tet on systolic blood pressure, heart weight, and collagen content Tet markedly decreased systolic arterial pressure in SHR (-34.2 %)[12], RHR (-41 %)[13], and DOCA-salt HR[15] (-45.6 %), absolute cardiac mass and left ventricular weight were reduced, the left ventricular wet weight to body weight ratios (LVW/BW) were also reduced by -22.6 % in SHR, -22.5 % in RHR, and -47.8 % in DOCA-salt RH by Tet, and -46.5 % by enalapril in RHR. These results indicated that Tet regressed cardiac mass in three models. The inhibitory degree in SHR was similar with RHR but much higher in DOCA-salt model. Tet decreased collagen content of LVH in SHR by 27 %, and in hypertrophic thoracic aorta Tet decreased it by 23 %. Tet decreased hydroxyproline content in LVH of RHR by 28 %. It indicated that prolonged treatment with Tet might markedly reduce blood pressure, inhibit regress left ventrticular hypertrophy, and decrease collagen content of cardiac mass on three models of hypertensive rats. That means Tet reversed remodeling in hypertrophied heart of hypertension. Kobrin[16] indicated that nitrendipine 10 mg/kg twice daily for 3 weeks in SHR decreased SBP (-27 %), LVW/BW (-5 %), HW/BW (-12.6 %) (LV: left ventricle; BW: body weight; HM: heart mass). Why regression of cardiac mass by nitrendipine was lesser than that of Tet? I suggest that the time of treatment was too short in nitrendipine treatment or other mechanism may exist. Pegram[17] showed that 3 weeks treatment of SHR with either methyldopa or clonidine significantly and similarly reduced mean

Tab 1. Effects of tetrandrine on systolic blood pressure (SBP) and cardiac hypertrophy in SHR, RHR, DOCA-salt HR rats. *n*=8. Mean±SD. $^{c}P<0.01$ *vs* normal. $^{f}P<0.01$ *vs* LVH.

Model	Group	SBP/kPa			Cardiac hypertrophy/mg·g^{-1}		
		Before	After	±%	LVWW/BW	RVWW/BW	VSWW/BW
SHR	Normal	15.0±1.4	15.7±1.3	+4.6 %	1.79±0.23	0.85±0.11	
	LVH	26.6±1.3^{c}	26.9±1.4^{c}	+1.1 %	2.61±0.27^{c}	1.05±0.06	
	LVH+Tet	26.3±1.5^{c}	17.3±1.3^{f}	-34.2 %	2.02±0.08^{f}	0.98±0.07	
RHR	Sham-operated	15.0±0.8	14.9±0.7	-0.6 %	1.80±0.15	0.53±0.15	
	LVH	25.5±2.4^{c}	26±4^{c}	+2.7 %	2.84±0.33^{c}	0.58±0.19	
	LVH+Tet	24.9±1.6^{c}	14.7±1.6^{f}	-41 %	2.20±0.26^{f}	0.59±0.07	
	LVH+Ena	25.8±2.2^{c}	13.8±1.8^{f}	-46.5 %	2.0±0.5^{f}	0.52±0.05	
DOCA-salt HR	Normal	13.7±0.5	13.4±0.9	-1.8 %	1.20±0.06	0.5±0.1	0.7±0.1
	LVH	26.8±0.6^{c}	26.4±1.1^{c}	-4.2 %	2.30±0.10^{c}	0.9±0.1^{c}	0.9±0.1^{c}
	LVH+Tet	27.2±0.6^{c}	14.8±0.8^{f}	-45.6 %	1.20±0.10^{f}	0.6±0.1^{f}	0.7±0.1^{f}

LVH: left ventricular hypertrophy. BW: body weight. LVWW: left ventricular wet weight; RVWW: right ventricular wet weight; VSWW: ventricular septa wet weight. Tetrandine 50 mg·kg^{-1}·d^{-1} ig for 9 weeks. Enalapril 6 mg·kg^{-1}·d^{-1} ig for 9 weeks.

arterial pressure (-11 % and -12 % respectively). Methyldopa, but not clonidine, reduced cardiac mass and HW/BW in SHR. Vasodilator hydralazine decreased mean arterial pressure (-19 %) in SHR but not cardiac mass. Hydralazine elevated HW/BW in WKY rats. Although anti-hypertensive effect of diltiazen was weaker than methyldopa in SHR[18], but it reduced HW/BW to a similar degree as methyldopa. These results indicated that the regression of hypertrophy following antihypertension therapy was neither solely dependent upon SBP nor suppression of adrenergic system. Leenen reported that minoxidil decreased mean blood pressure (MBP) markedly in RHR[19]. In contrast, enalapril caused a rapid and persistent normalization of MBP. LV and RV weight increased markedly after 3-5 weeks and left ventricular internal dimensions increased after 1-2 weeks by minoxidil treatment. Thus we could suggest that special structure of drug may produce special effect to regress cardiac mass. Tet might be an excellence drug to reduce cardiac mass, which was associated with lowering calcium overload. In a large multicenter clinical study, amlodipine 5-10 mg daily produced a mean decrease of 20 % after 16 weeks and 43 % after 42 weeks in LV mass index as compared with baseline, and fosinopril 20 mg daily reduced LV mass index by 16 % at 12th week[20,21]. The decrease in left ventricular mass was essentially caused by reduction of ventricular thickness, and free right ventricular wall thickness was also attenuated by both drugs. Tet reversed cardiac mass in SHR to a similar degree as amlodipine in patients and it was superior to other anti-hypertensive drugs (Tab 1 and 2).

Tab 2. Effects of tetrandrine on contents of collagen or hydroxyproline in SHR and RHR. *n*=6. Mean±SD. $^{c}P<0.01$ *vs* normal or sham-operated. $^{f}P<0.01$ *vs* LVH.

Model	Group	Contents of collagen/% of dry weight		
		LV	RV	Thoracic aorta
SHR	Normal	3.0±0.4	5.1±0.5	25.6±0.3
	LVH	4.43±0.22^{c}	5.2±0.4	36±4^{c}
	LVH+Tet	3.24±0.08^{f}	5.1±0.3	27.8±2.2^{f}
		Hydroxyproline/mg·g^{-1} dry weight		
RHR	Sham-operated	3.6±0.4		
	LVH	5.9±0.3^{c}		
	LVH+Tet	4.3±0.9^{f}		
	LVH+Ena	3.6±0.6^{f}		

Tetrandine 50 mg·kg^{-1}·d^{-1} ig for 9 weeks. Enalapril 6 mg·kg^{-1}·d^{-1} ig for 9 weeks.

Effects of Tet on haemodynamic changes and myocardial compliance of three model rats[12,13,22] The cardiac function and haemodynamic changes of LVH after Tet treatment for 9 weeks were shown in Tab 3.

Tab 3. Effects of tetrandrine on cardiac function and haemodynamics of isolated working heart with left ventricular hypertrophy in SHR ,RHR and DOCA-salt HR models. *n*=6 or 8. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* normal or sham-operated. $^{e}P<0.05$, $^{f}P<0.01$ *vs* LVH.

Model	Group	HR (beat/min)	LVSP (kPa)	LVEDP (kPa)	$+dp/dt_{max}$ ($kPa \cdot s^{-1}$)	$-dp/dt_{max}$ ($kPa \cdot s^{-1}$)	T value (ms)	AF/HWW (mL/min)	CF/HWW (mL/min)	CO/HWW (mL/min)
SHR	Normal	202±33	15.7±1.7	0.44±0.1	490±56	384±67	6.8±2	24±1.5	7.4±0.6	31.4±2.0
	LVH	162±35	11.6±1.4^{b}	1.47±0.5^{b}	451±139^{b}	163±52^{c}	17.5±1.4^{c}	14.9±1.7^{b}	5.5±0.5^{c}	20.4±3.1^{b}
	LVH+Tet	157±36	14.9±2.7^{e}	0.71±0.2^{e}	473±104^{e}	357±75^{f}	7.8±1.2^{f}	20.5±3.3^{f}	7.3±1.1^{f}	28±5^{d}
RHR	Sham-operated	220±14	15.2±0.5	0.52±0.33	543±17	396±24	6.7±2 .0	22.8±2.8	8.6±0.9	31±4
	LVH	203±13	12.9±0.8^{c}	1.06±0.37^{b}	440±19^{c}	289±15^{c}	16.9±1.7^{c}	16.4±1.3^{c}	6.1±0.7^{c}	22.5±1.9^{c}
	LVH+Tet	198±15	14.9±0.9^{e}	0.5±0.12^{e}	486±18^{f}	389±16^{f}	8.9±2.7^{f}	22.2±1.8^{f}	7.7±0.3^{f}	29.3±1.9^{f}
	LVH+Ena	218±14	15.7±1.9^{e}	0.4±0.4^{e}	585±51^{f}	399±29^{f}	8.8±2.3^{f}	29±5^{f}	9.4±0.5^{f}	38±7^{f}
DOCA-salt HR	Normal	220±15	15.7±1.7	0.54±0.12	560±26	370±32	6.8±1.9	43±1.7	13±2	56.0±0.9
	LVH	208±13	8.8±0.8^{c}	1.56±0.23^{c}	327±33^{c}	107±12^{c}	18±1.0 ^{c}	15.9±2.0^{c}	5.2±1.7^{c}	21.9±1.3^{c}
	LVH+Tet	222±11	15.0±0.7^{f}	0.77±0.10^{f}	540±61^{f}	335±32^{f}	7.6±0.9^{f}	32.0±2.0^{f}	10.3±1.3^{f}	42.8±1.7^{f}

The isolated working hearts were prepared 24 h after the last dose of drug. HR: heart rates; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure; $\pm dp/dt_{max}$: maximum rate of rise or fall of left ventricular pressure; T value: time constant of isovolumic diastolic pressure decay. HWW: heart wet weight. AF: aortic folw; CF: coronary flow; CO: cardiac output.

Tet treatment for 9 weeks decreased CF/HWW and CO/HWW in SHR (-26 % and -33.7 %), RHR (-29 % and -28 %), DOCA-salt HR models (-60 % and -61 %). It indicated that Tet recovered coronary and cardiac output flow in LVH especially in DOCA-salt HR. Diastolic function of working heart, as $-dp/dt_{max}$, LVEDP, and T value were improved more significantly than that of contractile function of LVH such as $+dp/dt_{max}$ and LVSP. That means diastolic function was more obviously impaired than that contractile function in early LVH. $-dp/dt_{max}$ was reduced by 58 %, while LVEDP and T value were increased by 234 % and 157 % in LVH of SHR compared with normal control. After Tet treatment for 9 weeks, $-dp/dt_{max}$ increased by 119 %, LVEDP and T value decreased by 52 % and 55 % compared with SHR, close to normal control. The changes of contractile and diastolic function were similar to SHR in both other models. Both LV compliance[23,24] (left ventricular diastolic pressure-volume curve, LV P-V curve) and LV stiffness constant[22] were other good parameters to reflect the diastolic function. LVDP (left ventricular developed pressure) represented LV mechanical function (starling). Compliance of LVH was significantly less than that of normal and Tet-treated groups in SHR[12], RHR[13,24] and DOCA-salt HR[22] models. LV P-V curve in LVH was shifted toward the left and down in Tet-treated group *vs* LVH group. LV stiffness constant of LVH was markedly lower after Tet treatment in DOCA-salt HR model, $P<0.01$[15]. These results showed that Tet could markedly improve diastolic function of left ventricle during LVH. Tet increased LVDP that was reduced in SHR[12], RHR[24], DOCA-salt HR[22] models, $P<0.01$ indicating that Tet could not only improve diastolic function but also enhance cardiac contractive function in LVH. Nitrendipine[16] makedly decreased cardiac mass, MBP, and total peripheral resistance in SHR without affecting cardiac output. Methyldopa[17] treatment for 3 weeks in SHR reduced cardiac mass, MBP, and total peripheral resistance but increase heart rate and lowered cardiac output. Clonidine reduced MBP to the same degree as methyldopa in SHR but it reduced heart rate yet without change in cardiac mass and cardiac output. Prolonged treatment of SHR with hydralazine also significantly decreased MAP while significantly increasing cardiac output and heart rate without affecting cardiac mass. So Kobrin[16] indicated that drugs regressed cardiac mass in rats through mechanisms that may be dissociated from their haemodynamic changes. Hydralazine[1], which did not attenuate LV hypertrophy but normalized blood pressure in SHR, is able to hinder the development of heart failure associated with advanced myocardial damage and detrimental chamber and interstitial remodeling. Irbesartan[25] (Ang II type–I receptor blocker) decreased systemic vascular resistance and

attenuated local expression of the rennin-angiotensin system but did not prevent adverse LV chamber and cardiomyocytes remodeling and did not improve LV function in early myocardial adaptive phase of mitral regurgitation in the dog either. Despite different effects of each drug, that Tet reduced BP and cardiac mass, improved diastolic and systolic cardiac function, and increased coronary flow and cardiac output in three hypertensive models was always consistent with them.

Effects of Tet on intracellular free calcium and Ca^{2+} content of cardiac mitochondria in LVH Ventricular myocytes and fibroblast cells isolated from normal and hypertrophied myocardium of SHR were loaded with Fura-2/AM 5 μmol/L and $[Ca^{2+}]_i$ was measured using AR-CM-MIC cations measurement system (SPEX Coroperation, USA) with computer software DM3000[27]. The resting $[Ca^{2+}]_i$[12] in hypertrophic cells was 72 % higher in SHR than that of normal myocyte [(150±21) *vs* (87±4) nmol/L], but in Tet-treated group it was (108±19) nmol/L, which was 28 % lower than that of SHR. Tet inhibited KCl 20, 40 mmol/L and NE 10, 20 μmol/L induced increase of $[Ca^{2+}]_i$ elevation in ventricular myocytes of SHR (Tab 4).

Tab 4. Effects of tetrandrine on resting $[Ca^{2+}]_i$ and KCl, NE-induced $[Ca^{2+}]_i$ elevation in myocytes of normal and SHR rats. *n*=6. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* before. $^fP<0.01$ *vs* LVH.

Groups	$[Ca^{2+}]_i$/nmol·L^{-1}				
	Before (resting)	KCl/mmol·L^{-1}		NE/μmol·L^{-1}	
		20	40	10	20
Normal	87±4	98±10^b	144±8^c	125±13^c	178±16^c
LVH	150±21	212±35^c	273±59^c	210±29^c	291±15^c
LVH+Tet	108±19	147±18^{cf}	187±20^{cf}	139±20^{cf}	161±27^{cf}

In isolated fibroblast cells[12] from normal myocardium, the resting $[Ca^{2+}]_i$ in SHR was was 67 % higher than that of normal ones, and KCl 40 mmol/L induced $[Ca^{2+}]_i$ elevation by 74 %. In Tet-treated group KCl 40 mmol/L induced only 9 % increase of $[Ca^{2+}]_i$. These results showed that Tet decreased $[Ca^{2+}]_i$ elevation by inhibiting calcium influx (Tab 5).

Ca^{2+} in mitochondria of the left ventricular cells in Tet-treated group was significantly lower than normal group[15], which was similar with myocytes and fibroblast cells.

Effects of Tet on ATPase and myosin ATPase activity in hypertrophied myocytes in SHR and RHR Na^+,K^+-ATPase activity on membrane and sarcoplasmic reticulum was reduced by 60 % and 48 % in LVH of SHR compared with normal control, but increased by 116 % and 62 % after Tet treatment compared with SHR. Ca^{2+}-ATPase activity on sarcoplasmic reticulum was reduced by 29 % *vs* normal control, but increased by 46 % by Tet *vs* SHR[12]. Tet greatly increased Na^+, K^+-ATPase and Ca^{2+}-ATPase activity in LVH of RHR[28,29].

Tab 5. Effects of tetrandrine on resting $[Ca^{2+}]_i$ and KCl-induced $[Ca^{2+}]_i$ elevation in fibroblasts. *n*=6. Mean±SD. $^cP<0.01$ *vs* before. $^fP<0.01$ *vs* LVH.

Groups	$[Ca^{2+}]_i$/nmol·L^{-1}	
	Before (resting)	KCl 40 mmol/L
Normal	105±19^f	168±18^{cf}
LVH	176±17	292±36^c
LVH+Tet	122±19^f	185±20^{cf}

There are two isoforms of heavy chain of myosin, *ie*, α-MHC (adult type) and β-MHC (fetal type). Myosin isoenzyme V_1 is the ββ homodimer with the highest ATPase activity; V_3 is the αα homodimer with the lowest ATPase activity; while V_2 is believed to be the αβ heterodimer. V_1 is the predominant isoenzyme in adult and V_3 is mainly in fetal animal heart. Myosin isoenzyme may converted from V_1 to V_3 when LVH developed. The decrease of contractility of hypertrophied myocardium was closely associated with the change of myosin isoform. In our study, myosin ATPase activity[29] in LVH was reduced by 55.7 % in RHR *vs* sham-operated group, in Tet-treated group it was increased by 60 % *vs* RHR. That means Tet could improve cardic function by regulating balance between V_3 and V_1. Cerivastatin[26] (hydroxymethylglutaryl coenzyme A reductase inhibitor) improves LV remodeling and LV systolic, diastolic function in rats with chronic heart failure after experimental myocardial infarction. It was associated with an attenuated expression of fetal genes such as β-CMHC, a collagen I gene expression that was inhibited to retard progression of chronic heart failure (Tab 6).

Effects of Tet on properties of [^{3}H](+)-isradipine binding to left ventricle in LVH[15] [^{3}H](+)-isradipine binding was monitored as described by Glassmann and Ferry[31]. In left ventricular hyper-

Tab 6. Effects of tetrandrine on Na^+,K^+-ATPase activity on membrane and ATPase activities on sarcoplasmic reticulum (SR) in hypertrophied myocardium in SHR. *n*=6. Mean±SD. $^cP<0.01$ *vs* normal. $^fP<0.01$ *vs* LVH.

Groups	ATPase activity/mmol·h^{-1}·g^{-1} protein			
	Myocardial membrane	Sarcoplasmic reticulum		
	Na^+,K^+-ATPase	Na^+,K^+-ATPase	Ca^{2+}-ATPase	Mg^{2+}-ATPase
Normal	19.1±2.5	16.3±2.1	25±5	24±6
LVH	7.6±2.2^c	8.5±2.3^c	18±4^c	29±4
LVH+Tet	16.4±1.0^f	14±3^f	26±5^f	28±6

trophic rats, B_{max} of dihydropyridine binding sites in left ventricular membranes was significantly increased in DOCA-salt hypertensive rats as compared with that in the normal rats (Tab 7, $P<0.01$). In Tet–treated group, it was significantly decreased by 45 % compared with DOCA-salt hypertensive rats (Tab 7, $P<0.01$). The total number of dihydropyridine receptors per left ventricle in Tet–treated group was decreased by 55 % than DOCA-salt hypertensive rats. There was no marked difference in the K_d values between each group.

Tab 7. Effects of tetrandrine on characteristics of dihydropyridine binding sites in left ventricular hypertrophic membranes from DOCA-salt hypertensive rats. *n*=8. Mean±SD. $^cP<0.01$ *vs* normal. $^fP<0.01$ *vs* LVH.

Group	K_d /nmol·L^{-1}	B_{max}/pmol·g^{-1} protein	TNR/ pmol per LV
Normal	0.15±0.01	404±18	3.9±0.4
LVH	0.17±0.01	835±47^c	9.93±0.21^c
LVH+Tet	0.17±0.01	461±11^f	4.6±0.5^f

TNR: the total number of receptors; LVH: left ventricular hypertrophy; LVH+Tet: Tet treated LVH; normal: normotensive rats.

We evaluated the effects of chronic treatment with Tet on cardiac remodeling using three left ventriclar hypertrophied hypertensive models. Prolonged treatment with Tet may markedly reduce blood pressure and regress left ventriclar hypertrophy, *ie* improve remodeling. It suggested that Tet-induced cardiac remodeling regression may be partially associated with blood pressure control in early phase. However control of arterial pressure may not always result in regression of remodeling. For example, minoxidil markedly decreased BP but significantly increased LV and RV weight after 3-5 weeks of treatment in RHR[19]. Neurohumoral factors were important in development of remodeling, including Ca^{2+} signal transduction. Ca^{2+} plays a pivotal role in myocardial signal transduction and as an second messenger. Tet as a calcium antagonist reduced density of Ca^{2+} channels binding sites in LVH and inhibited intracellular Ca^{2+} overload and KCl, NE-induced $[Ca^{2+}]_i$ elevation in hypertrophic myocytes. Tet also improved energy metabolism by increasing Na^+, K^+-ATPase, Ca^{2+}-ATPase, and myosin ATPase activity. Tet could improve cardiac contractility and diastolic function, cause regression of left ventricular mass, and decrease collagen content in left ventricular remodeling. It can be concluded that Tet is an excellent drug to treat hypertension and LVH.

EFFECTS OF TET ON VASCULAR REMODELING

Chronic sustained hypertension is typically characterized by an elevated peripheral vascular resistance in both experimental animals and human beings. The increased vascular resistence is caused by dynamic and structural changes of the small and main arteries. In hypertension changes in small artery structure are basically divided into 2 kinds[32]: (1) inward eutrophic remodeling, in which outer and lumen diameters are decreased, media/lumen ratio is increased, and cross-sectional area of the madia is unaltered; and (2) hypertrophic remodeling, in which the media thickens to encroach on the lumen, resulting in increased media cross-sectional area and media/lumen ratio. Growth of smooth muscle cells, apoptosis, inflammation, and fibrosis contributed to vascular remodeling in hypertension. Vascular fibrosis entails accumulation of collagen, fibronectin, and other extracellular matrix components in the vessel wall and is an important aspect in extracellular matrix remodeling in hypertension. Chronic vasoconstriction may lead to embedding of the contracted vessel structure in a remodeled extracellular matrix contributing to inward remodeling of the blood vessel as smooth muscle cells are re-arranged around a smaller lumen. The resulting remodeling of small arteries may initially be adaptive but gradually become maladaptive and do harm to organ function, and at last induce cardiovascular complications of hypertension.

Angiotensin-converting enzyme inhibitors and cal-

cium antagonists have beneficial effects on regression of vascular remodeling. Intracellular calcium was an important factor in smooth muscle cell proliferation, vascular contraction, and the release of endothelium-dependent factors. So a lot of agents or factors, such as wall stress, Ang II, and catecholamines altered structure and function of arteries in part through their effect on intracellular calcium. Agents that affect intracellular calcium pathways directly (calcium antagonists) or indirectly (ACEI) may have vascular protective effects, partly by regressing vascular remodeling and by correcting endothelial dysfunction. Here I will review our study about effect and mechanism of Tet on regression of vascular remodeling in two-kindey-one-clip (2K1C) goldblott renovascular hypertrophic rats (RHR).

Regression of vascular remodeling in RHR by Tet[32,33] The wet weight of aorta, caudal artery, and femoral artery of RHR (18 weeks after 2K1C operation) were greater than those of sham-operated rats. Media thickness in abdomen aorta was increased by 32.1 % *vs* sham-operated rats. The cross section of media of tail artery was increased by 41.4 % [(5.8±1.2) μm^2 *vs* (4.1±1.3) μm^2] . In Tet and Ena-treated rats it was (4.9±0.9) μm^2 and (4.2±1.1) μm^2 respectively. The media thickness and media to lumen ratio (M/L ratio) of coronary, renal, and mesenteric arterioles were significantly increased, which were more greater in arterioles with vessel diameter less than 70 μm. For vessel with diameter less than 70 μm and between 70 to 150 μm, M/L ratio was increased significantly about 70.3 % and 53.4 % in renal arterioles and by 57.5 % and 44 % in coronary arterioles. There were no marked changes in numbers of smooth muscle cells in most vessel wall, except in renal arteriols, where the number of smooth muscles cell were increased by about 37 % (P<0.05 *vs* sham). The results suggest that it is mainly a "hypertrophic" and "rearrangement" remodeling in vessels of RHR. In Tet and enalapril-treated rats, all the changes were ameliorated. Tet and Ena have beneficial effect on regression of vascular remodeling in RHR. Enalapril[4] and losartan equally (but not hydralazine) attenuated increased wall/lumen ratio and LVW/BW ratio induced by *L*-NAME. Both drugs modulated different intracellular signaling pathways, inhibiting P70S6K (70-kDa S6 kinase) or ERK (extracellular signal-regulated protein kinase) respectively, to elicit reduction of cardiac hypertrophy induced by chronic inhibition of NO synthesis *in vivo* (Tab 8).

Regression of vessels resistance and microvascular reactivity in RHR by Tet[34] Auricular microvessels of rats exhibited a significant decreased response to hot stimulus and an increased response to cold stimulus at 10 and 16 weeks after 2K1C operation. It has a prolonged period to return to normal blood flow after withdrawal of both stimuli. A significant elevation of serum ACE activity and a decline of serum nitric oxide level were observed. Tet could markedly moderate abnormal reactivity to vasoconstrictive and vasodilative stimuli in RHR[34].

Chen *et al*[35] reports that Tet given before ischemia may be involved in reduction of microvascular permeability in stunned myocardium from rats. The contraction and relaxation of resistance vessels to stimuli were investigated in mesenteric and tail arteries in RHR[36]. Compared with sham-operated rats, isolated perfused mesenteric arterioles and tail artery exhibited an enhanced reactivity to NE-induced vasoconstriction with rise in maximum vasoconstriction and decline in vasodilation stimulated by ACh and sodium nitroprusside (SNP) respectively. The sensitivity of tail artery to methylene blue (Met), an inhibitor of guanylate cyclase was greatly reduced. The amplitude of elevated pressure after removal of endothelium was attenuated. Those changes above were greatly reversed by Tet. The results suggest that in RHR, the reactivity of microvessels and vessels resistance to vasoconstrictive stimuli are increased, whereas the reactivity to both endothelium-depedent or independent vasorelaxative stimuli are decreased. Tet 50 $mg \cdot kg^{-1} \cdot d^{-1}$ for 9 weeks markedly reverse those changes by preserving endothelium function and guanylate cyclase.

The mechanisms related to vascular remodeling in RHR and pharmacological effects of Tet[32].

Ultrastructure of aorta suggested that aortic VSMC (AVSMC) in RHR have transferred from contractile phenotype to synthetic phenotype. Compared with sham, AVSMC from RHR showed a higher proliferative property (A=0.88±0.04 in RHR *vs* 0.758±0.026 in sham, P<0.05). Tet reduced NE or AngII 0.l μmol/L-stimulated AVSMC proliferation. Tet 0.1-10 μmol/L *in vitro* induced a concentration-dependent depression in [^{3}H]-thymidine incorporation stimulated by NE or AngII in AVSMCs from either RHR or sham rats. It provides an evidence of increased reactivity to NE or Ang II of AVSMC in RHR. Tet inhibited proliferation and DNA synthesis in AVSMCs and depressed the susceptibility of AVSMC to AngII and NE[37]. Calcium antagonist, cilnidipine blocked both L-and N-type calcium chan-

Tab 8. Effects of tetrandrine and enalapril (Ena) on media width and media to lumen ratio in arterioles of RHR. n=6. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* sham-operated control. ^{e}P<0.05, ^{f}P<0.01 *vs* age-matched untreated RHR.

Vessels	Sham	RHR	Tet	Ena
Media width/μm				
70 μm>Ø				
Coronary	6.9±0.9	10.5±2.7^{c}	7.2±1.1^{e}	7.6±2.5
Renal	7.4±1.2	12±3^{c}	8.8±1.1^{e}	7.3±1.0^{f}
70 μm<Ø<150 μm				
Coronary	15.3±2.8	18.6±0.7^{b}	17.1±1.6	16.4±2.1^{e}
Renal	17.2±2.4	24±4^{c}	19.1±2.2^{e}	18.7±2.4^{f}
Mesenteric	24.2±2.1	28±4	19.6±1.9^{f}	18±5^{f}
Media to lumen ratio (M/L)				
70 μm>Ø				
Coronary	0.22±0.06	0.35±0.04^{c}	0.26±0.08^{e}	0.23±0.04^{f}
Renal	0.34±0.07	0.59±0.03^{c}	0.44±0.06^{e}	0.37±0.12^{f}
70 μm<Ø<150 μm				
Coronary	0.26±0.07	0.38±0.07^{b}	0.30±0.05^{e}	0.28±0.05
Renal	0.37±0.04	0.56±0.04^{c}	0.31±0.09^{f}	0.39±0.04
Mesenteric	0.39±0.09	0.57±0.11^{b}	0.30±0.10^{f}	0.32±0.08

Ø: diameter; 70 μm>Ø means diameter less than 70 μm; 70 μm<Ø<150 μm means diameter between 70 to 150 μm. Tet 50 mg·kg^{-1}·d^{-1} and Ena 6 mg·kg^{-1}·d^{-1} treated for 9 weeks from the 9th week after 2K1C operation.

nels of VSMC from SHR. Cilnidipine 1 and 10 mmol/L exert its antiproliferative effect by antagonizing calcium channels and inhibiting basal DNA synthesis and expression of TGF-βl mRNA in VSMC from SHR[38].

Apotosis, programmed cell death with minimal membrane disruption and inflammation, counters cell proliferation and regulates developmental growth. It was reported to be both increased and decreased in hypertension in different tissues including blood vessels. Is apoptosis involved in development of aorta and tail arteries remodeling in RHR and is the inhibitory effect of Tet on proliferation mediated by apoptosis? TUNEL, [^{3}H]-TdR incorporation, and flow cytometry demonstrated an increased apoptosis rate of AVSMC in RHR with apoptotic index of 2.1±1.2 in aorta and 1.3±1.4 in tail artery, whereas in sham it was 0.3±0.2 and 0.2± 0.5 respectively. It was increased significantly in Tet- and enalapril-treated RHR. Tet directly induced apoptosis of cultured AVSMC from RHR by inhibiting FCS, TGF-$\beta_{1,}$ and TNF-α mRNA expression. The results suggested that Tet-induced AVSMCs apoptosis at least partly was related with the regression of vascular remodeling[32] (Tab 9, 10).

Tab 9. Effect of Tet and enalapril (Ena) on apoptosis index detected by TUNEL method in RHR. n=5. Mean±SD. ^{b}P<0.05 *vs* Sham.

Groups	Aorta	Tail artery
Sham-operated	0.3±0.2	0.2±0.5
RHR	2.1±1.2^{b}	1.3±1.4^{b}
RHR+Tet	3.5±2.8^{b}	2.2±1.7^{b}
RHR+Ena	4.2±2.1^{b}	2.6±2.1^{b}

Collagen deposition is existed in RHR and whether it is affected by Tet? Compared with sham, hydroxyproline content in aorta was increased by 36.6 % in RHR, while it was reduced by 18.7 % and 19.9 % in Tet- and enalapril-treated RHR (P<0.01 *vs* RHR), respectively. In cultured AVSMCs separated from sham and RHR, Tet 0.1-10 μmol/L *in vitro* induced a concentration-dependent depression in [^{3}H]-proline incorporation stimulated by NE or Ang II in AVSMCs from either sham or RHR. Those results suggest that depression of collagen synthesis and deposition in arteries by Tet

Tab 10. Effect of tetrandrine(Tet) and enalapril (Ena) on apoptosis ratio and necrosis ratio in cultured AVSMCs. *n*=4. Mean±SD. [c] *P*<0.01 *vs* control. [f] *P*<0.01 *vs* sham.

Groups	Apoptosis/%		Necrosis/%	
	Sham	RHR	Sham	RHR
Control (0.1 % FCS)	6.6±2.9	4.1±0.7	4.4±1.0	5.8±0.6
Tet (10 μmol/L)	28±3[c]	28±4[c]	4.3±1.2	6.6±0.9
Ena (10 μmol/L)	16±3[c]	30±3[cf]	4.2±1.1	6.3±1.1

contributed to regression of vascular remodeling[39].

To clarify the role of Tet in reversing vascular remodeling and regulating function of endothelium and nitric oxide (NO), NO level in serum and NO synthesis in isolated and cultured aorta were measured. Compared with sham control NO level in serum was reduced in RHR. NO production by cultured aorta with or without ACh stimulation were significantly decreased in RHR. In cultured AVSMC in normal rats, SNP inhibited cell proliferation and induced apoptosis. But in AVSMC from RHR, the above effects were weaker than in normal rats. The proliferative response to *L*-NAME of AVSMC from RHR was also reduced. Tet could reverse these changes. The results suggested that there was a function deficiency in endothelium in RHR, with a decreased reactivity to NO in smooth muscle cells. Tet protected endothelial function in RHR and sensitized reactivity of VSMC of RHR to NO, which were beneficial to regression of vascular remodeling[32].

Tet reverses vascular media thickness and collagen deposition and improves the reactivity of microvessels and decreases resistance vessels. The mechanisms of Tet in vascular remodeling regression are as follows: 1) Inhibiting phenotype transfer of VSMC in hypertension, decreasing VSMC's response to mitogenic stimuli such as NE and Ang II; 2) Sensitizing VSMC to pro-apoptosis stimuli; 3) Improving the endothelial function; 4) Enhancing anti-proliferation effect of NO on VSMC.

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