

• 研究简报 •

小檗碱对肾性高血压心肌肥厚模型大鼠左心室重塑的影响

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Effect of berberine on left ventricular remodeling in renovascular hypertensive rats

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Abstract: The purpose of this study is to evaluate the effects and the underline mechanisms of berberine on the cardiac function and left ventricular remodeling in rats with renovascular hypertension. The renovascular hypertensive model was established by the two-kidney, two-clip (2K2C) method in Sprague-Dawley (SD) rats. Two weeks after surgery, all the operated SD rats were randomly assigned into four groups: ① renovascular hypertensive model group; ② berberine 5 mg·kg⁻¹ group; ③ berberine 10 mg·kg⁻¹ group; ④ captopril 45 mg·kg⁻¹ group; and the sham operated rats were used as control. Four weeks after the drugs were administered, the cardiac function was assessed. The ratios of heart weight to body weight (HW/BW), left ventricular weight to body weight (LVW/BW) and right ventricular weight to body weight (RVW/BW) were compared between groups. Coronal sections of the left ventricular tissue (LV) were prepared for paraffin sections, picosirius red and HE staining was performed. The left ventricular wall thickness (LVWT), interventricular septal thickness (IVST), the parameters of myocardial fibrosis indicated by interstitial collagen volume fraction (ICVF) and perivascular collagen area (PVCA) were assessed. Nitric oxide (NO), adenosine cyclophosphate (cAMP) and guanosine cyclophosphate (cGMP) concentrations of left ventricular tissue were measured. Berberine 5 mg·kg⁻¹ and 10 mg·kg⁻¹ increased the left ventricular $\pm dp/dt_{max}$ and HR. Berberine 10 mg·kg⁻¹ decreased HW/BW and LVW/BW. The image analysis showed that both 5 and 10 mg·kg⁻¹ of berberine decreased LVWT, ICVF and PVCA, while increased the NO and cAMP contents in left ventricular tissue. Berberine could improve cardiac contractility of 2K2C model rats, and inhibit left ventricular remodeling especially myocardial fibrosis in renovascular hypertension rats. And such effects may partially associate with the increased NO and cAMP content in left ventricular tissue.

Key words: berberine; 2K2C model; left ventricular remodeling

左心室重塑是高血压导致的心脏的重要病理改变之一,并最终导致心脏舒缩功能障碍,甚至发生心

衰^[1],因此预防与逆转左心室重塑已成为治疗高血压的重要目标。已有研究表明,一些种类的降压药如血管紧张素转换酶抑制剂、血管紧张素受体拮抗剂、醛固酮受体拮抗剂及钙拮抗剂能够有效逆转心脏重塑^[2-5]。然而有些降压药如米诺地尔,却可加重心肌肥厚和/或心肌缺血^[6]。小檗碱(berberine,

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Ber)为黄连等植物中的主要有效成分,属异喹啉类生物碱,已证明其对心血管系统有十分广泛的作用,如降压、抗心律失常、改善顽固性心衰等^[7]。近年有资料显示,小檗碱对实验性动物心肌肥厚具有保护作用^[8-10]。为进一步探讨小檗碱的抗心肌肥厚作用及相关机制,本文采用双肾双夹致肾性高血压大鼠模型,观察小檗碱对左心室重塑及心功能的影响。

材料和方法

动物 雄性 Sprague-Dawley (SD)大鼠,体重 220~240 g,北京维通利华实验动物技术有限公司提供,许可证 SCXK(京)2002-2003。

药物与试剂 小檗碱、卡托普利及天狼星红染料 (sirius red)均购于 Sigma 公司 (USA);羧甲基纤维素钠 (CMC-Na)购于北京化学试剂公司。一氧化氮 (NO)试剂盒及总蛋白测定试剂盒 (南京建成生物工程研究所);cAMP 和 cGMP 放免测定药盒 (上海中医药大学同位素室)。其余试剂为市售分析纯。

双肾双夹模型的建立及分组给药 参照 Sattar 等^[11]的文献,采用双肾双夹法制备大鼠心肌肥厚模型。腹腔注射戊巴比妥钠 ($40 \text{ mg} \cdot \text{kg}^{-1}$)麻醉,无菌条件下开腹,分离两侧肾动脉后,用 0 号丝线将 0.26 mm 的渔线和肾动脉一起结扎,然后抽出渔线,缝合腹壁,腹腔注射庆大霉素 ($40\,000 \text{ u} \cdot \text{mL}^{-1}$, 0.1 mL/只)抗感染。假手术组动物开腹后分离肾动脉但不结扎,其余步骤与手术动物相同。

术后 2 周,根据尾血压 (BESN-II 多通道动物无创测压系统,南京德赛生物技术有限公司)水平将手术动物分为假手术组、模型组、小檗碱 5 及 $10 \text{ mg} \cdot \text{kg}^{-1}$ 组、卡托普利 $45 \text{ mg} \cdot \text{kg}^{-1}$ 组。将药物混悬于 0.5% CMC-Na,按 $10 \text{ mL} \cdot \text{kg}^{-1}$ 每日一次灌胃给予,假手术组和模型组同时给予等体积 0.5% CMC-Na,连续给药 4 周。

心功能测定 给药结束后,给大鼠腹腔注射戊巴比妥钠 ($45 \text{ mg} \cdot \text{kg}^{-1}$)麻醉并仰位固定,分离右侧颈总动脉。将 18G 静脉套管 (MALAYSIA)由颈总动脉插管至左心室,连接 Bridge Amplifier 经 MacLab/400 生物信号记录仪 (澳大利亚)记录左心室压力变化,并记录左心室收缩压 (left ventricular systolic pressure, LVSP)、左心室舒张压 (left ventricular end-diastolic pressure, LVEDP)、左心室最大收缩舒张速率 ($\pm \text{dp}/\text{dt}_{\text{max}}$)、心率 (heart rate, HR)等。

心重指数测定 称取大鼠体重 (body weight, BW)后处死,开胸迅速摘取心脏,先剪去周围大血管,吸干血液,称全心重量 (heart weight, HW);再剪去心房,称取左心室重量 (left ventricular weight, LVW)、右心室重量 (right ventricular weight, RVW)。计算全心重量指数 (HW/BW)、左心室重量指数 (LVW/BW)及右心室重量指数 (RVW/BW),考察心脏肥厚的程度。

HE 染色 取左心室赤道面心肌环,用 10% 甲醛液固定,石蜡切片,厚度为 $5 \mu\text{m}$,进行 HE 染色,用 Image ProPlus 5.0 图像分析系统测量左心室壁厚度 (left ventricular wall thickness, LVWT)及室间隔厚度 (interventricular septal thickness, IVST)。

苦味酸天狼星红 (picric acid-sirius red) 染色 取石蜡切片,在二甲苯中彻底脱蜡至水,在 0.1% 苦味酸天狼星红溶液中染色 1 h,流动自来水冲洗 5 min,用 Harris 苏木精复染,在各级乙醇内脱水,二甲苯透明,树脂封片。在偏振光显微镜下 (Nikon, 日本),用 10 倍物镜观察,采用 Brilla 等^[12]描述的方法,用 Image ProPlus 5.0 图像分析系统测量心肌间质胶原容积分数 (interstitial collagen volume fraction, ICVF) 和心肌血管周围胶原面积 (perivascular collagen area, PVCA)。ICVF = 心肌间质胶原面积 / 所测视野面积。PVCA = 血管周围胶原面积 / 血管腔面积。每张切片各随机分析 6 个视野,并取均值。

左心室肌组织 NO, cAMP 和 cGMP 含量测定 取左心室肌,准确称取重量后,加入组织块重量 4 倍的生理盐水进行匀浆 (冰浴),制备成 20% 组织匀浆。再以 $1\,800 \text{ r} \cdot \text{min}^{-1}$ 离心 6 min,取适量上清液,用硝酸还原酶法测定 NO 含量,并用考马斯亮蓝法进行蛋白定量,NO 含量以 $\mu\text{mol} \cdot \text{mg}^{-1}$ (protein) 表示。取 20% 组织匀浆, $2\,000 \text{ r} \cdot \text{min}^{-1}$ 离心 10 min,取上清液 $100 \mu\text{L}$,按 cAMP 和 cGMP 放免测定药盒说明书操作。

统计学分析 数据以 $\bar{x} \pm s$ 表示,采用 SPSS11.5 统计软件包,以 *t* 检验进行组间比较。

结果

1 小檗碱对双肾双夹模型大鼠心功能的影响

结扎双侧肾动脉 6 周后,模型组大鼠左心室舒张末期压力呈升高趋势,舒张压最大变化速率及心率呈下降趋势,但与假手术组相比未见显著差异。而小檗碱可显著加快 $\pm \text{dp}/\text{dt}_{\text{max}}$ 及 HR,增强了此模型大鼠的心肌收缩能力 (表 1)。

Table 1 Effect of berberine on cardiac function in 2K2C rats

Group	Dose /mg• kg ⁻¹	n	LVSP /mmHg	LVEDP /mmHg	(+) dp/dt _{max} (mmHg• s ⁻¹)	(-) dp/dt _{max} (mmHg• s ⁻¹)	HR /beats• min ⁻¹
Model		11	180 ±26	0.09 ±3.02	8 520 ±918	- 6 800 ±1 266	440 ±30
Sham		10	167 ±16	- 1.24 ±2.90	8 460 ±816	- 7 155 ±705	469 ±29
Berberine	5	9	198 ±27	- 3.86 ±2.72	9 669 ±1 165*	- 8 229 ±962*	467 ±12*
	10	9	190 ±29	- 2.50 ±2.19	9 405 ±756*	- 8 055 ±901*	482 ±12*
Captopril	45	9	183 ±18	- 3.08 ±3.12	9 120 ±742	- 7 520 ±977	475 ±21*

LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end-diastolic pressure; + dp/dt_{max}: The maximum rate of contraction; - dp/dt_{max}: The maximum rate of relaxation; HR: Heart rate. Model group: 2K2C operated rats without any treatment; Sham group: Sham operated rats without any treatment; all the other groups administered with drugs (ig) orally two weeks after 2K2C operation for four weeks. The cardiac function was assessed at the end of study. $\bar{x} \pm s$. * $P < 0.05$ vs model group

Table 2 Effect of berberine on the ratios of heart weight to body weight in 2K2C rats

Group	Dose /mg• kg ⁻¹	n	HW /BW (g• kg ⁻¹)	LVW /BW (g• kg ⁻¹)	RVW /BW (g• kg ⁻¹)
Model		14	3.19 ±0.23	2.31 ±0.21	0.55 ±0.03
Sham		13	2.76 ±0.14***	1.94 ±0.11***	0.49 ±0.04**
Berberine	5	12	3.09 ±0.32	2.21 ±0.28	0.53 ±0.04
	10	11	2.88 ±0.25*	2.07 ±0.18*	0.50 ±0.06*
Captopril	45	12	2.95 ±0.29*	2.09 ±0.25*	0.51 ±0.06*

HW /BW: Heart weight/body weight; LVW /BW: Left ventricular weight/body weight; RVW /BW: Right ventricular weight/body weight. $\bar{x} \pm s$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs model group

2 小檗碱对双肾双夹模型大鼠心重指数的影响

与假手术组相比,模型组大鼠心重指数显著增加,连续给药4周,小檗碱 10 mg• kg⁻¹及卡托普利 45 mg• kg⁻¹可显著降低模型大鼠的 HW /BW, LVW /BW 及 RVW /BW,表明小檗碱能抑制双肾双夹模型大鼠的心肌肥厚(表 2)。

3 小檗碱对双肾双夹模型大鼠左心室壁厚度及室间隔厚度的影响

与假手术组相比,模型组大鼠 LVWT及 IVST均显著增加。小檗碱 10 mg• kg⁻¹可显著减少 LVWT(表 3)。

Table 3 Effect of berberine on LVWT and IVST in 2K2C rats

Group	Dose /mg• kg ⁻¹	n	LVWT/mm	IVST/mm
Model		11	3.67 ±0.41	2.99 ±0.57
Sham		10	2.98 ±0.51***	2.48 ±0.27**
Berberine	5	9	3.55 ±0.46	3.02 ±0.46
	10	9	3.35 ±0.35*	3.17 ±0.35
Captopril	45	9	3.56 ±0.24	2.97 ±0.48

LVWT: Left ventricular wall thickness; IVST: Interventricular septal thickness. $\bar{x} \pm s$. * $P < 0.05$, ** $P < 0.01$ vs model group

4 小檗碱对双肾双夹模型大鼠左心室胶原分布的影响

苦味酸-天狼星红染色后,在偏振光显微镜下观察显示:假手术组大鼠心肌间质及血管周围红色和黄色的 I型胶原纤维较少,且分布均匀;模型组 I型胶原明显增多,且汇集成条索状;小檗碱干预后, I型胶原增生有所改善。半定量分析显示,小檗碱能显著降低 ICVF和 PVCA(表 4,图 1)。

Table 4 Effect of berberine on ICVF and PVCA in 2K2C rats

Group	Dose /mg• kg ⁻¹	n	ICVF/%	PVCA
Model		11	0.81 ±0.32	2.14 ±1.01
Sham		10	0.42 ±0.16***	1.21 ±0.34***
Berberine	5	9	0.63 ±0.32	1.12 ±0.49*
	10	9	0.47 ±0.25***	1.31 ±0.43**
Captopril	45	9	0.53 ±0.26**	1.25 ±0.41**

ICVF: Interstitial collagen volume fraction; PVCA: Perivascular collagen area. $\bar{x} \pm s$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs model group

5 小檗碱对双肾双夹模型大鼠左心室肌组织 NO, cAMP和 cGMP含量的影响

与假手术组比,模型组大鼠左心室肌组织 NO

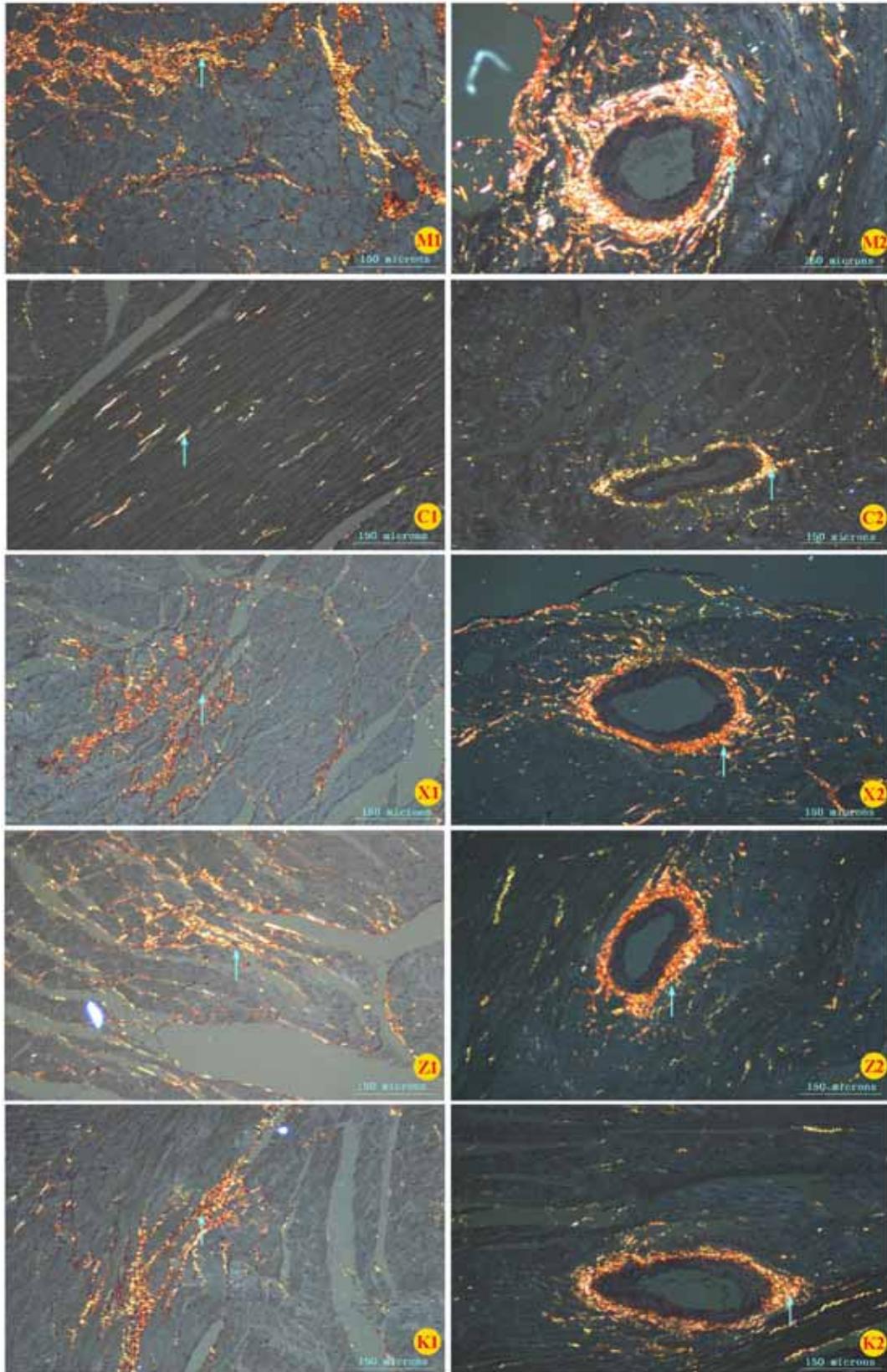


Figure 1 Effect of berberine on collagen content in rats by picric acid-sirius red staining. M: Model group; C: Sham group; X: Berberine $5 \text{ mg} \cdot \text{kg}^{-1}$; Z: Berberine $10 \text{ mg} \cdot \text{kg}^{-1}$; K: Captopril $45 \text{ mg} \cdot \text{kg}^{-1}$. 1: Myocardial interstitial collagen; 2: Perivascular collagen. Magnification $\times 100$

Table 5 Effect of berberine on NO, cAMP and cGMP contents in left ventricular tissue in 2K2C rats

Group	Dose /mg • kg ⁻¹	n	NO	cAMP	cGMP	cAMP/cGMP
			/μmol • g ⁻¹ (protein)	/pmol • g ⁻¹ (tissue)	/pmol • g ⁻¹ (tissue)	
Model		11	0.72 ± 0.35	81 ± 14	16.71 ± 1.88	4.87 ± 0.71
Sham	-	10	1.26 ± 0.31 ^{**}	99 ± 27	20.92 ± 1.97 ^{***}	5.06 ± 1.52
Berberine	5	9	1.24 ± 0.53 [*]	108 ± 18 ^{**}	16.69 ± 1.92	6.08 ± 0.57 [*]
	10	9	1.50 ± 0.61 ^{**}	104 ± 24 [*]	16.38 ± 2.31	7.93 ± 4.26 [*]
Captopril	45	9	1.43 ± 0.69 [*]	109 ± 26 [*]	17.28 ± 4.04	6.59 ± 2.61

$\bar{x} \pm s$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs model group

含量显著降低,小檗碱及卡托普利均显著增加心肌 NO 含量(表 5)。与假手术组比,模型组大鼠左心室肌组织中的 cAMP 及 cGMP 的含量均降低,小檗碱及卡托普利可显著升高 cAMP 含量,使 cAMP/cGMP 显著升高(表 5)。

讨论

高血压心脏结构、功能重建的特点和机制以及抗高血压药物对其逆转作用已成为近年来研究的热点之一。高血压引起的心脏结构重建主要为左心室肥厚(LVH),是心脏对慢性压力和容量超负荷的适应性变化,其特征包括:左心室内径增大和/或室壁增厚、心脏重量增加、左室容积增大、心肌僵硬度增加等。根据左室壁相对厚度及左室重量指数将左室构型分为 4 种:正常构型;向心性重构;向心性肥厚^[13];离心性肥厚。心肌肥厚的显微结构变化包括:心肌细胞肥大、间质纤维化及冠脉微循环的结构改变,其中心肌纤维化是心功能障碍的主要病理基础^[14]。因此抑制心肌纤维化对保护心功能具有重要意义。

本研究发现,结扎大鼠双侧肾动脉 6 周后,模型大鼠全心重指数及左心室重量指数升高,左室壁及室间隔增厚,心腔缩小,呈向心性肥厚的特征;同时心肌间质及血管周围胶原纤维增生明显,且部分血管管壁增厚,管腔缩小。给予小檗碱治疗 4 周,使 2K2C 心肌肥厚模型全心重量指数、左心室重量指数下降,左心室壁的厚度下降;左心室心肌间质胶原容积分数和血管周围胶原面积减小,与模型组比较有显著差异,且疗效与目前被大多数学者公认为具有预防和逆转心肌纤维化作用的 ACEI 卡托普利相近。说明小檗碱可以减轻左心室肥厚,抑制左室的纤维化,具有一定的抑制肾性高血压大鼠左心室重构的作用。文献^[15]报道肾性高血压模型大鼠 6 周时心功能已经出现损害,本试验结果亦显示模型大鼠左心室舒张末压呈升高趋势,而舒张压最大变

化速率呈下降趋势,显示心室舒张功能受损,与文献报道相符。心肌舒张性能受损较早可能与模型左室壁及室间隔厚度增加,以及左心室心肌间质的纤维化导致心肌顺应性降低有关。小檗碱可降低模型大鼠的左心室壁厚度,减轻心肌间质与血管周围纤维化,有助于提高模型大鼠左心室 $\pm dp/dt_{max}$,增强心肌收缩能力,并改善心室舒张功能。另外,心肌收缩能力还受到神经-体液因素影响^[16],小檗碱通过增加模型大鼠左心室肌组织中 cAMP 含量使心肌收缩力增强,与早期研究结果^[7]相符。

NO 是一种重要的血管舒张因子,近年研究发现,NO 不但能够直接抑制心脏成纤维细胞增殖,降低纤维连接蛋白、I 型胶原和 III 型胶原基因表达、促进间质胶原降解,而且能够通过抑制肾素-血管紧张素-醛固酮系统激活,降低血压等间接途径抑制心肌纤维化^[17-19]。因此,NO 是抑制心肌纤维化的重要因素之一。本研究结果显示,小檗碱可显著提高肾性高血压心肌肥厚模型大鼠左心室肌组织的 NO 含量,可能是其抑制左心室纤维化的作用环节之一。

References

- [1] Tsotetsi QJ, Woodiwiss AJ, Netjhardt M, et al. Attenuation of cardiac failure, dilatation, damage, and detrimental interstitial remodeling without regression of hypertrophy in hypertensive rats [J]. Hypertension, 2001, 38: 846 - 851.
- [2] Yamamoto K, Mano T, Yoshida J, et al. ACE inhibitor and angiotensin II type 1 receptor blocker differently regulate ventricular fibrosis in hypertensive diastolic heart failure [J]. J Hypertens, 2005, 23: 393 - 400.
- [3] Matsumura K, Fujii K, Oniki H, et al. Role of aldosterone in left ventricular hypertrophy in hypertension [J]. Am J Hypertens, 2006, 19: 13 - 18.
- [4] Chen H, Zhang L, Zhu D, et al. Effects of diltiazem on myocardial remodeling of left ventricle in renal hypertensive rats [J]. J Fourth Mil Med Univ (第四军医大学学报), 2005, 26: 594 - 598.
- [5] Willeite RN, Anderson KM, Nelson AH, et al.

- Enrasentan improves survival, limits left ventricular remodeling, and preserves myocardial performance in hypertensive cardiac hypertrophy and dysfunction [J]. *J Cardiovasc Pharmacol*, 2001, 38: 606 - 617.
- [6] Sica DA, Gehr TW. Direct vasodilators and their role in hypertension management in inoxidil [J]. *J Clin Hypertens (Greenwich)*, 2001, 3: 110 - 114.
- [7] Cao X. Berberine's application on treating cardiovascular diseases [J]. *Integr Tradit Chin West Med Prac Crit Care Med (中西医结合实用临床急救)*, 1994, 1: 44 - 46.
- [8] Yang J, Zhou ZY, Xu JG. Protective effect of berberine on cardiac hypertrophy induced by L-thyroxine in rats [J]. *J Sichuan Univ (Med Sci Ed) (四川大学学报医学版)*, 2004, 35: 223 - 225.
- [9] Hong Y, Hui SS, Chan BT, et al. Effect of berberine on catecholamine levels in rats with experimental cardiac hypertrophy [J]. *Life Sci*, 2003, 72: 2499 - 2507.
- [10] Hong Y, Hui SS, Chan TY, et al. Effect of berberine on regression of pressure-overload induced cardiac hypertrophy in rats [J]. *Am J Chin Med*, 2002, 30: 589 - 599.
- [11] Sattar MA, Yusuf AP, Gan EK, et al. Acute renal failure in 2K2C Goldblatt hypertensive rats during antihypertensive therapy: comparison of an angiotensin AT1 receptor antagonist and clonidine analogues [J]. *J Auton Pharmacol*, 2000, 20: 297 - 304.
- [12] Brilla CG, Reams GP, Maisch B, et al. Renin-angiotensin system and myocardial fibrosis in hypertension: regulation of the myocardial collagen matrix [J]. *Eur Heart J*, 1993, 14: 57 - 61.
- [13] Ganau A, Devereux RB, Roman M, et al. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension [J]. *J Am Coll Cardiol*, 1992, 19: 1559 - 1560.
- [14] Frohlich ED. State of the art lecture. Risk mechanisms in hypertensive heart disease [J]. *Hypertension*, 1999, 34: 782 - 789.
- [15] Chen YG. Renal hypertensive LV hypertrophy and mechanical changes in myocardium after the reverse with drugs [J]. *Chin J Cardiac Funct (心功能杂志)*, 1998, 10: 221 - 224.
- [16] Regitz-Zagrosek V, Nertrampf R, Steffen C, et al. Myocardial cyclic AMP and norepinephrine content in human heart failure [J]. *Eur Heart J*, 1994, 15: 7 - 13.
- [17] Ritchie RH, Schiebinger RJ, LaPointe MC, et al. Angiotensin II-induced hypertrophy of adult rat cardiomyocytes is blocked by nitric oxide [J]. *Am J Physiol*, 1998, 275: H1370 - H1374.
- [18] Kim NN, Villegas S, Summerour SR, et al. Regulation of cardiac fibroblast extracellular matrix production by bradykinin and nitric oxide [J]. *J Mol Cell Cardiol*, 1999, 31: 457 - 466.
- [19] Takizawa T, Gu M, Chobanian AV, et al. Effect of nitric oxide on DNA replication induced by angiotensin II in rat cardiac fibroblasts [J]. *Hypertension*, 1997, 30: 1035 - 1040.