

缬沙坦对不同时期高血压大鼠血浆和心肌肾素-血管紧张素系统及左心室重塑的影响

彭永平 李俭春 江时森 陈锐华

(南京军区南京总医院心脏科, 南京 210002)

主题词 血管紧张素 ① 醛固酮; 受体; 拮抗剂; 高血压; 胶原; 大鼠

摘要 从受体水平阻断肾素-血管紧张素系统的生物学效应来探讨血管紧张素 ① 和醛固酮对高血压大鼠心肌肥厚及纤维化的作用, 将二肾一夹型高血压大鼠分为高血压治疗组和高血压对照组, 高血压治疗组在术后第16周通过饮水给予缬沙坦($10\text{ }\mu\text{g/g}\cdot\text{d}$), 高血压对照组和假手术组不给药。术后第16周、20周及28周分别处死大鼠, 测定血压、心脏和左心室重量、血浆和心肌血管紧张素 ① 及醛固酮浓度、左心室重量指数、心肌胶原含量及胶原容积分数。结果发现, 高血压对照组大鼠血浆和心肌血管紧张素 ① 及醛固酮浓度、左心室重量指数、心肌胶原浓度及胶原容积分数均显著高于假手术组($P < 0.05$ 或 0.005); 与高血压对照组比较, 高血压治疗组血浆血管紧张素 ① 浓度明显增高, 血压、左心室重量指数、血浆醛固酮浓度、心肌胶原浓度及胶原容积分数则显著降低($P < 0.05$), 且主要是iv型胶原减少。结果提示, 肾血管性高血压大鼠左心室重塑与心肌血管紧张素 ① 和醛固酮浓度密切相关, 血管紧张素 ① 型受体拮抗剂缬沙坦可以阻断血管紧张素 ① 的病理生理作用, 抑制醛固酮的释放, 逆转左心室重塑, 心脏局部血管紧张素 ① 的生物学效应可能主要是血管紧张素 ① 型受体所介导。

Role of Valsartan in Plasma and Myocardial Renin-Angiotensin System and Left Ventricular Remodeling in Different Phases of Hypertensive Rats

PENG Yong- Ping, LI Jian- Chun, JIAN Shi- Sen and CHEN Rui- Hua

(Department of Cardiology, General Hospital of Nanjing of People's Liberation Army, Nanjing 210002, China)

MeSH Angiotensin ① Aldosterone; Receptors; Antagonists; Hypertension; Collagen; Rats

ABSTRACT **Aim** To investigate the role of angiotensin ① (Ang ①) and aldosterone (Ald) in myocardial hypertrophy and fibrosis in hypertensive rats by blocking renin-angiotensin system at receptor level. **Methods** Thirteen 2 kidney-1 clip (2K1C) hypertensive rats began to be administered valsartan in the drinking water ($10\text{ }\mu\text{g/g}\cdot\text{d}$) at the sixteenth week after operation. Nineteen 2K1C rats were used as untreated controls. Nineteen sham operated rats were also used as controls. Systolic blood pressure (SBP), left ventricular weight index (LVWI), plasma and myocardial Ang ① /Ald concentration, myocardial collagen concentration (MCC) and collagen volume fraction (CVF) were measured when three groups rats were killed at the sixteenth week, the twentieth week and the twenty-eighth week after operation.

Results All measured facts in 2K1C hypertensive group were significantly higher than in sham operated group ($P < 0.05$ or 0.005). Compared with age-matched 2K1C group, plasma Ang ① concentration was higher significantly ($P < 0.05$), but SBP, LVWI, MCC, CVF and plasma Ald concentration in treated group decreased significantly ($P < 0.05$). **Conclusions** Left ventricular myocardial remodeling in renovascular hypertensive rats was closely related to myocardial Ang ① and Ald concentration. Ang ① (AT_1) receptor antagonist valsartan can block the pathophysiological effects of Ang ① , inhibit Ald secretion and reverse left ventricular remodeling (mainly decrease deposition of type iv collagen). It suggests that AT_1 receptors may mediate the biological effects of Ang ① in local tissue.

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近年来, 大量研究表明血管紧张素 ① (angiotensin ① , Ang ①) 在心肌肥厚和间质胶原增生等组织结构改变 (即心肌重塑, remodeling) 中可能起关键作用, 但详尽机制不甚清楚^[1]。临床及实验已证实血管紧张素转化酶 (angiotensin converting enzyme, ACE) 抑制剂可逆转心肌肥厚, 但 ACE 不是 Ang ① 生成的唯一途径, 还存在糜酶等其它途径。Ang ① 型

(AT_1) 受体拮抗剂从受体位点直接阻断 Ang ① 的作用环节可能在逆转心肌重塑中有良好的前景^[2]。本研究拟观察二肾一夹型高血压大鼠心肌肥厚、胶原增生的发展过程与心肌局部 Ang ① 和醛固酮 (aldosterone, Ald) 的关系, 新型非杂环类和高度特异的 AT_1 受体拮抗剂缬沙坦对不同时期高血压大鼠血浆、心肌 Ang ① 和醛固酮的影响以及逆转心肌肥厚、纤维化的作用。

1 材料和方法

1.1 实验动物及分组

4 周龄雄性近交系 Wistar 大鼠 60 只(购自中国科学院上海实验动物中心), 体重 180~ 220 g, 20% 乌拉坦 1 mg/g, 腹腔注射麻醉。在无菌条件下, 游离左肾动脉并放置内径为 0.25 mm 不锈钢夹, 右肾动脉不触及, 术后 4 周收缩压 ≥ 20 kPa 或收缩压大于术前血压的 3 个标准差并高于 18.67 kPa 则确定模型建立^[3](共 32 只)。随机分为两组: 高血压对照组(2K1C 组) 19 只; 高血压治疗组(Val+ 2K1C 组) 13 只, 术后第 16 周开始通过饮水方式给予缬沙坦 (10 $\mu\text{g/g}\cdot\text{d}$); 另外假手术组(sham 组) 18 只, 手术方法同上, 但放置内夹后即刻取出。

1.2 血压测定

在清醒状态下, 用大鼠电脑血压心率仪(上海市高血压研究所研制) 采用尾袖法测定大鼠收缩压, 取 3 次血压的均值, 术前及术后每 2 周测 1 次。

1.3 血浆及组织样品设备

术后第 16 周、20 周及 28 周分期处死大鼠。处死大鼠前抽取动脉血, 5 mL 加入预处理管, -4°C 离心, 取上清, -30°C 保存, 供测 Ang II 2 mL 加入肝素抗凝管, 离心, 取血浆, -30°C 保存, 供测醛固酮含量。大鼠处死后迅速摘取心脏, 用预冷生理盐水清洗, 分离左心室, 滤纸吸干, 称重, 左心室重量指数(left ventricular weight index, LVWI) 为左心室重量与体重之比; 左心室一部分称重后加入 0.5 mol/L 乙酸 5 mL, 煮沸 15 min, 冷却后匀浆, 4°C 离心, 取上清, -30°C 保存, 测 Ang II 和醛固酮含量; 另取一块心肌组织称重后, 放入具塞试管中, 加入 6 mol/L HCl 2 mL, 置 $120^{\circ}\text{C}\sim 130^{\circ}\text{C}$ 烘箱 24 h, 冷却后调 pH=6, 滤液供测心肌胶原含量(myocardial collagen concentration, MCC); 一部分(心尖部) 置于 10% 中性福尔马林液,

供组织化学染色。

1.4 血管紧张素 II 和醛固酮含量测定

血浆和心肌 Ang II 和醛固酮含量测定采用放射免疫法, 药盒购自北京北方免疫研究所, 按药盒要求操作。采用氯胺 T 法测定心肌羟脯氨酸含量, 羟脯氨酸浓度乘以 8.2 即为胶原浓度^[4]。胶原特殊染色采用苦味酸天狼猩红偏振光法^[5]。光镜下用计算机辅助方法测定胶原容积分数(collagen volume fraction, CVF), 偏振光下观察 iv 型和 III 型胶原。

1.5 统计学处理

数据以均数 \pm 标准差($\bar{x} \pm s$) 表示, 两组间比较用 t 检验; 多组间比较用方差分析; 相关分析用直线相关。

2 结果

2.1 治疗前后收缩压的变化

高血压对照组大鼠治疗后收缩压显著高于假手术组($P < 0.05$)。高血压治疗组大鼠收缩压逐渐下降, 至治疗后第 4 周降至较平稳的水平, 显著低于高血压对照组($P < 0.05$), 与假手术组无显著性差异(表 1, Table 1)。

2.2 各组血浆和心肌肾素-血管紧张素系统及左心室重塑相关参数比较

高血压对照组 LVWI、血浆和心肌 Ang II 及醛固酮浓度、MCC 和 CVF 均显著高于假手术组($P < 0.05$), 其中 MCC 和 CVF 呈进行性增加。高血压治疗组大鼠血浆 Ang II 显著增加, 而 LVWI、血浆及心肌醛固酮浓度、MCC 和 CVF 皆下降, 尤以后二者更显著(表 1, Table 1)。相关分析显示, 高血压对照组大鼠心肌胶原浓度与心肌 Ang II 含量呈显著正相关($r = 0.8737, P < 0.001$), 与心肌醛固酮含量也呈正相关($r = 0.7140, P < 0.002$)。

表 1. 各组大鼠左心室重量指数、血浆和心肌血管紧张素 II 和醛固酮含量、心肌胶原含量及胶原容积分数的比较

Table 1. Changes of the plasma and myocardial Ang II Ald content, LVWI, MCC and CVF in rats ($\bar{x} \pm s$)

Index	the 16th week		the 20th week			the 28th week		
	Sham	2K1C	Sham	2K1C	Val+ 2K1C	Sham	2K1C	Val+ 2K1C
	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=7)	(n=7)
SBP (kPa)	15.7 \pm 0.9	27.7 \pm 1.4 ^b	16.1 \pm 0.6	26.4 \pm 0.8 ^a	18.0 \pm 0.3 ^c	16.0 \pm 0.7	25.8 \pm 1.1 ^a	17.3 \pm 0.6 ^c
LVWI (mg/g)	1.86 \pm 0.06	3.2 \pm 0.6 ^b	1.92 \pm 0.11	3.04 \pm 0.15 ^a	2.27 \pm 0.10 ^c	2.03 \pm 0.11	2.97 \pm 0.60 ^a	1.96 \pm 0.08 ^c
Plasma Ang II (ng/L)	169 \pm 27	401 \pm 122 ^b	174 \pm 3.5	440 \pm 69 ^a	968 \pm 140 ^{ac}	189 \pm 45	445 \pm 63 ^a	572 \pm 174 ^a
Plasma Ald (nmol/L)	0.89 \pm 0.06	1.77 \pm 0.30 ^b	0.87 \pm 0.14	1.6 \pm 0.4 ^a	1.09 \pm 0.22 ^c	0.76 \pm 0.14	1.81 \pm 0.09 ^a	0.56 \pm 0.13 ^{ac}
Myocardial Ang II (mg/L \cdot g wet weight)	5.5 \pm 0.9	16.5 \pm 2.7 ^b	7.6 \pm 1.6	15.7 \pm 1.9 ^a	14 \pm 4 ^a	7.7 \pm 1.2	13.3 \pm 2.9 ^a	15 \pm 4 ^a
Myocardial Ald (g/L \cdot g wet weight)	10.5 \pm 2.6	23 \pm 5 ^b	9.4 \pm 0.5	14.9 \pm 1.5 ^a	14 \pm 3 ^a	12.0 \pm 1.6	14.7 \pm 2.6	11.7 \pm 1.4
MCC (mg/g)	5.4 \pm 0.6	7.4 \pm 0.2 ^b	5.6 \pm 0.4	7.8 \pm 0.3 ^a	6.29 \pm 0.28 ^{ac}	5.58 \pm 0.20	8.8 \pm 0.4 ^a	6.9 \pm 0.7 ^{ac}
CVF (%)	1.09 \pm 0.12	2.01 \pm 0.23 ^b	1.25 \pm 0.25	2.27 \pm 0.17 ^a	1.31 \pm 0.12 ^c	1.33 \pm 0.30	3.14 \pm 0.37 ^a	1.88 \pm 0.27 ^c

a: $P < 0.05$, b: $P < 0.005$, compared with sham group; c: $P < 0.05$, compared with 2K1C group

2.3 病理形态分析

假手术组大鼠心肌壁内冠状小动脉外膜有少量胶原堆积,不向周围延伸,心肌间质有少量胶原纤维,以 iv 型(黄红色)胶原为主,Ⅲ型(绿色)胶原较少。高血压对照组大鼠心肌壁内冠状小脉外膜胶原异常沉积,并向周围延伸(即反应性纤维化),心肌间质胶原纤维增多增粗,呈栅栏状包裹心肌细胞,以 iv 型胶原为主,纤维呈螺旋状,双折光强;Ⅲ型胶原次之,双折光弱,后期胶原增生更显著。高血压治疗组大鼠总的胶原减少,iv 型和 Ⅲ型胶原沉积,长期作用更显著,提示缬沙坦主要减少 iv 型胶原纤维沉积。

3 讨论

2K1C Goldblatt 高血压大鼠是 Ang Ⅱ和醛固酮均增高且激活纤维化的理想实验模型,肾素-血管紧张素-醛固酮系统(renin-angiotensin-aldosterone system, RASS)在肾动脉钳夹 1 个月之内被激活,在第 2、3 个月期间逐渐减活^[6]。本实验结果显示,在术后第 16 周、20 周和 28 周 2K1C 大鼠血浆和心肌 Ang Ⅱ和醛固酮仍处于较稳定的高激活状态。左室肥厚明显,后期 LVWI 有减低趋势,而 MCC 呈进行性增加,提示高血压左室肥厚后期主要是心肌胶原的增生和间质结构的改变,以 iv 型胶原增生为主,Ⅲ型胶原次之。左心室的间质纤维化开始于冠状动脉周围,然后沿伸至邻近间质空间,最后形成替代性纤维化微疤痕,与 Nicoletti 等^[7]研究结果相似。高血压性左室重塑可能是多因素的,机械力学的刺激(如心室压力增高及冠状动脉灌注压升高等)和激素(诸如 Ang Ⅱ、醛固酮、肽及 TGF- β_1 等)协同作用促进心肌细胞蛋白质合成增加和非心肌细胞(主要是成纤维细胞)增殖,间质胶原合成增多^[8]。本实验结果表明,新型非杂环类高度特异的 AT₁ 受体拮抗剂缬沙坦不仅能降低血压,而且能减少醛固酮的释放,逆转心肌肥厚,明显减少胶原沉积(主要是减少 iv 型胶原纤维沉积),其长期作用更显著。而 LVWI、血浆和心肌醛固酮水平接近正常对照,但 MCC 不能完全逆转,其原因可能是胶原清除是很慢的过程, MCC 后期回降,稍高于高血压对照组,而心肌局部 Ang Ⅱ保持较为稳定状态,提示机体可能存在负反馈

调节机制。有学者报告,小剂量氯沙坦(AT₁受体拮抗剂)在不降低血压的情况下可逆转左心室肥厚,而胍苯哒嗪虽降低收缩压却没能逆转心肌肥厚,提示 Ang Ⅱ致心肌肥厚作用是独立于血液动力学的关键因素^[9]。本研究提示,AT₁受体可能介导 Ang Ⅱ致心肌细胞肥大、成纤维细胞增殖、间质胶原沉积作用和促进醛固酮释放。有资料表明 AT₂受体可能介导抗血管平滑肌细胞增殖和诱导细胞凋亡(apoptosis)的作用^[2,10],AT₁受体拮抗剂阻断 AT₁受体位点,使 Ang Ⅱ浓度升高,促进 Ang Ⅱ与 AT₂受体的结合,这在心肌重塑的逆转中可能起一定作用,然而确切机制有待进一步研究。

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