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Attenuation of Left Ventricular Hypertrophy by Angiotensin II Receptor Antagonist in Rats

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Abstract Recent major concern has been focused on the direct effect of locally produced angiotensin II in cardiac tissue on cardiac hypertrophy. We assessed the role of angiotensin II on the development of left ventricular (LV) pressure-overload hypertrophy by using a newly developed angiotensin II receptor antagonist, TCV-116. We administered 0.3 mg/kg/day (low dose;LD), 3.0 mg/kg/day (high dose;HD) of angiotensin II receptor antagonist (TCV-116) and vehicle (aortic constriction (AC)) to abdominal aortic banding rats, and vehicle to sham-operated rats (Sham) as a control. Four weeks after aortic banding, LV hypertrophy was observed as evidenced by increases in wall thickness and LV weight. Both in the LD and HD groups, end-diastolic wall thickness decreased to a similar extent compared with that in the AC group. In addition, the HD treatment prevented an increase in end-diastolic internal dimension associated with significant reduction of peak wall stress. These additional effects observed in the HD group were not accompanied by a further reduction of LV pressure. On the other hand, dP/dt at a developed LV pressure of 40mmHg in the HD group was suppressed to a greater extent than that in the LD group. In conclusion, TCV-116 attenuated cardiac hypertrophy probably by an inhibition of local angiotensin II in cardiac tissue. Although LV contractility was reduced by TCV-116, LV pump function was preserved due to a concomitant reduction of LV wall stress.

Key words: Rat, Wall stress, Pump function

Introduction

The fact that hypertension results in left ventricular (LV) hypertrophy is a well recognized adaptation of the myocardium to reduce wall stress. However, chronic pressure overload may be associated with accelerated deterioration of cardiac function and increased incidence of heart failure. Therefore, reversal or prevention of LV hypertrophy is often viewed as a desirable goal of antihypertensive drug therapy.

Recent studies have been focused on the role of the local renin-angiotensin system in the formation of LV hypertrophy For example, angiotensin II(Ang II) acts as a growth factor on cardiac myocytes[1,2] and angiotensin converting enzyme inhibitors induce regression or prevention of LV hypertrophy both in experimental animal models and in hypertensive patients[3-5]. On the other hand, Ang II is known to have a positive inotropic effect on the myocardium[6,7] and hence whether treatment by Ang II inhibition actually improves LV performance remains to be elucidated[8].

TCV-116, a recently introduced nonpeptide

antagonist to the type 1 Ang II receptor, inhibited noncompetitively the binding between Ang II and the type 1 Ang II receptor and is thought to be useful in clarifying the role of the renin-angiotensin system in development of LV hypertrophy.

The goals of the present study were 1) to assess the role of Ang II in the development of cardiac hypertrophy induced by abdominal aorta constriction; 2) to determine whether prevention of LV hypertrophy by Ang II inhibition is beneficial for cardiac function.

Materials and Methods

Male Wistar rats were obtained from laboratories (Japan SLC, Inc., Japan). Pressure overload hypertrophy was produced by constriction of the abdominal aorta in male Wistar rats (body weight; 140-170g) anesthetized with 3.6% chloral hydrate. The abdominal aorta was surgically isolated above the left renal artery and constricted by using the ligature-needle technique as described previously[9]. Sham-operated rats underwent a similar surgical procedure except for the aorta constriction. The animals were divided into four groups;untreated rats with aortic constriction (AC group), rats with aortic constriction and 0.3mg/kg per day of TCV -116 treatment (low dose;LD group), rats with aortic constriction and 3.0mg/kg per day (high dose;HD group), and untreated sham-operated rats (Sham group). Every treatment was administered once daily by oral gavage. The treatment was started a day before the surgical procedure and continued for 4 weeks. TCV-116 was provided by Takeda Chemical Industries, Ltd.

The rats were anesthetized (50mg/kg i.P. thiopental sodium) 24 hours after the last administration, and intubated and mechanically ventilated. The right carotid artery was exposed and cannulated with a 2Fr. microtip pressure transducer (model PR249, Millar Instruments, Houston, Texas). The microtip pressure transducer was then advanced into the LV for the evaluation of LV pressure and rate of pressure rise (dP/dt of LV pressure). Subsequently, thoracotomy via the third left intercostal space was performed to expose the heart. LV anterior wall thickness was measured by 20 MHz wall tracking module (WT-20, Crystal Biotech, Inc. Hopkinton, USA) attached to the mid portion of the epicardium of LV anterior wall with triggering peak (+) dP/dt of LV pressure. After the hemodynamic measurements, the heart was arrested by intravenous injection of 1 M KCl, and rapidly excised. The right ventricle free wall was trimmed away, and the LV was weighed. Hemodynamic data were stored on a magnetic tape (Sony Instrumentation Data recorder UN-61430) at tape speed of 9.5mm/sec. The analogue signals were digitized by a 12-bit A/D converter connected to a microcomputer (PC 9801 RA;NEC) at intervals of 1 msec and stored on disc. Ten consecutive cardiac cycles were sampled on each stage and averaged. (dP/dt) at an isovolumic developed pressure of 40mmHg [(dp/dt)/DP40] were measured or derived from the digital data of the isovolumic portion of the average LV pressure pulse. To calculate LV internal dimension and wall stress, we made the following assumptions[10]; a) the shape of the left ventricle is spherical, b) LV wall thickness is uniform at all portions of the left ventricle, c) specific gravity of the myocardial tissue is 1.06. We applied the equation of midwall fiber stress as the equation of wall stress. Midwall fiber stress(σ) was the circumferential stress at midwall for a sphere and calculated using the following equation[11].

LV midwall radius=(WT+D)/2

σ=(LV pressure×LV midwall radius)/(2×WT)

where D is internal dimension of the LV cavity,

WT is LV wall thickness

Statistical Analysis

Values are expressed as means ±SD. Significant differences between multiple groups were analyzed by analysis of variance. P values at 0.05 or less were considered statistically significant. This study was approved by Animal Care Committee of the School of Medicine, Yamaguchi University.

Results

LV weight in the AC group was significant-

ly greater than that in the Sham group. TCV -116 treatment reduced the LV weight in a dose-dependent manner (Table 1).

The parameters of hemodynamics are summarized in Table 2. In every three banding groups peak LV pressure was elevated as compared with that in the Sham group. Peak LV pressure in each treated groups was lower than that in the AC group, but no significant difference was observed between the treated groups. Heart rate and LV end -diastolic pressure did not differ among all groups. (dp/dt)/DP40 in the AC group was not different from that in the LD or Sham groups. On the other hand, (dp/dt)/DP40 in the HD group was suppressed as compared with that in the AC and LD groups.

LV geometry data are shown in Figure 1. LV end-diastolic wall thickness (Wed) was increased in the AC group, whereas the increment of Wed was severely attenuated in both treated groups (LD and HD). In the HD group, the increment in LV end-diastolic internal diameter (Ded) was also suppressed as compared with that in the LD group. Wed/Ded ratio, which is defined as an index of the concentricity of LV geometry, was greater in the AC group than in the Sham group, whereas normalized in both treated groups.

Although end-diastolic wall stress was not significantly different among all groups, peak wall stress was increased in the AC group reflecting the elevation of peak LV pressure, while normalized in the HD group. In the LD group, interestingly, peak wall stress remained at a high level despite the comparable reduction of LV pressure in the HD group. There was no difference in LV fractional shortening among all groups (Figure 2 and 3).

Discussion

The present study demonstrated that Ang

Table 1 Left ventricular weight and the ratio of left ventricular weight to body weight

Treatment	AC (n=9)	LD (n=9)	HD (n=9)	Sham (n=8)
LVW (mg)	718±67★ # §	580±65★ #	489±44★	408±30
LVW/BW (mg/g)	2.8±0.3★ # §	2.3±0.3★ #	2.1±0.2★	1.6±0.1

Values are expressed as means±SD.; LVW, Left Ventricular Weight; BW, Body Weight; AC, untreated rats with aortic constriction; LD, rats with aortic constriction and 0.3 mg/kg per day of TCV-116 treatment; HD, rats with aortic constriction and 3.0 mg/kg per day of TCV-116; Sham, sham-operated rats;
★ $p< 0.05$ vs. Sham, # $p<0.05$ vs. HD, § $p<0.05$ vs. LD.

Table 2 Hemodynamics

Treatment	AC (n=9)	LD (n=9)	HD (n=9)	Sham (n=8)
HR (beats/min)	420±45	421±47	423±51	427±28
Peak LVP (mmHg)	164±9 ★ # §	135±9★	137±12 ★	121±7
LVEDP (mmHg)	7±2	5±2	5±2	4±2
(dP/dt)/DP40 (sec^{-1})	121± 24 #	117± 22 #	95± 13	106 ±17

Values are expressed as means ± SD.; Peak LVP, maximal value of Left Ventricular Pressure; LVEDP, LV end-diastolic Pressure; Peak (+) dP/dt , maximal value of first derivative of LV pressure; (dP/dt)/DP40, dP/dt at an isovolumic developed pressure of 40 mmHg. ★ $p<0.05$ vs. Sham, # $p<0.05$ vs. HD, § $p<0.05$ vs. LD .

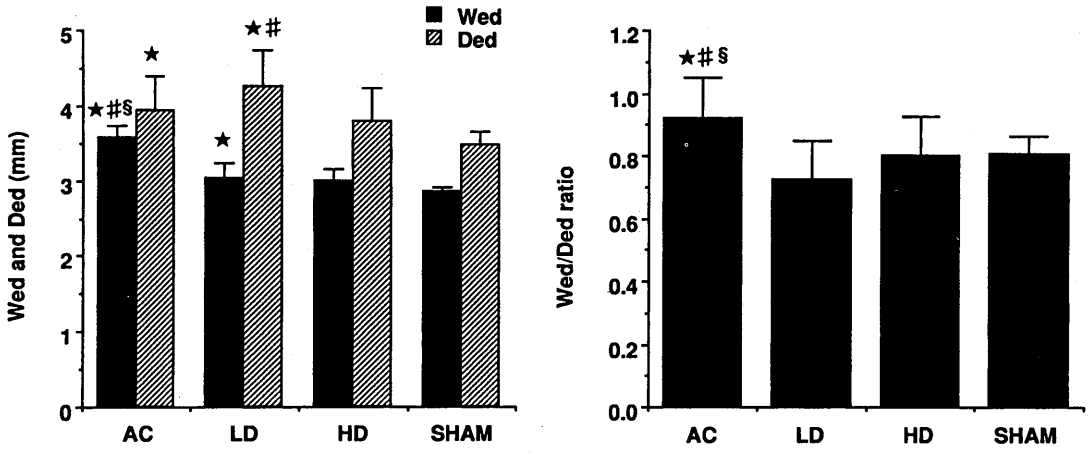

Figure 1 Bar graphs showing the effect of TCV-116 on left ventricular geometry in abdominal aorta constricted rats. Results are expressed as means ±SD. Wed, end-diastolic wall stress; Ded, end-diastolic internal dimension; AC, untreated rats with aortic constriction; LD, rats with aortic constriction with 0.3mg/kg per day of TCV-116; HD, rats with aortic constriction with 3.0mg/kg per day; Sham, sham-operated rats.
★ $p<0.05$ vs. Sham, # $p<0.05$ vs. HD, * $p<0.05$ vs. LD.

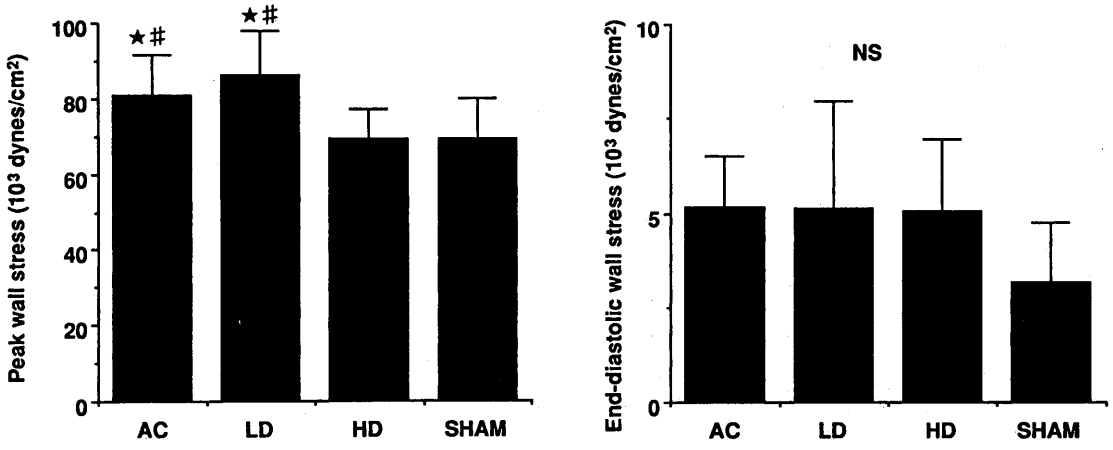

Figure 2 Bar graphs showing the effect of TCV-116 on peak wall stress and end-diastolic wall stress in abdominal aorta constricted rats. Results are expressed as means ± SD. AC, untreated rats with aortic constriction; LD, rats with aortic constriction with 0.3mg/kg per day of TCV-116; HD, rats with aortic constriction with 3.0 mg/kg per day; Sham, sham-operated rats.
★ $p<0.05$ vs. Sham, # $p<0.05$ vs. HD.

II receptor antagonist (TCV-116) prevented the pressure overload hypertrophy without proportional reduction of LV afterload. Although such prevention by TCV-116 suppressed LV contractility, LV pump function was preserved due to a concurrent reduction of LV wall stress.

Circulating Ang II is well known to mediate both arterial and venous vasoconstriction, resulting in elevation of preload and

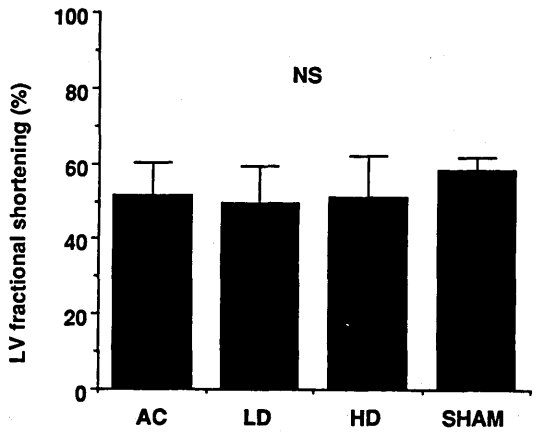

Figure 3 Bar graph showing the effect of TCV-116 on left ventricular fractional shortening. Results are expressed as means ±SD. No significant differences was observed among all groups. AC, untreated rats with aortic constriction; LD, rats with aortic constriction with 0.3mg/kg per day of TCV-116; HD, rats with aortic constriction with 3.0mg/kg per day; Sham, sham-operated rats.

afterload of the heart. Recent studies revealed that the Ang II receptor was localized in cardiac tissue[12,13], and several reports suggest a direct effect of Ang II on cardiac muscle growth response. Sadoshima et al.[14] showed that mechanical stretch caused a release of Ang II from cardiac myocyte and that Ang II acted as an initial mediator of the stretch-induced hypertrophic response. Kojima et al.[15] confirmed that Ang II receptor antagonist (TCV-116) in fact inhibited intracellular signaling of stretch mediated cardiomyocyte in vitro. In the present in vivo study, we demonstrated the significant prevention of pressure overload hypertrophy by a high dose of TCV-116. This preventive effect was associated with no additional reduction of peak LV pressure, suggesting that a local Ang II released from cardiac tissue, which can be blocked by TCV-116, might be responsible for pressure overload hypertrophy.

In the HD group, peak wall stress, which was increased in untreated abdominal aorta banding rats, was decreased to the same level as that in sham-control rats due to a normalization of wall thickness and end-diastolic internal dimension. Interestingly, however, in the LD group, peak wall stress remained at a high level despite the comparable reduction in LV pressure with the HD group. This finding suggests that insufficient dose of TCV-116 failed to reduce LV wall stress despite its significant vasodilating effect.

In pressure overload LV hypertrophy, systolic function is usually preserved at a compensatory state. In the present study, systolic function was preserved or even enhanced in untreated aortic banding group as evidenced by the tendency to an increase in (dP/dt)/DP40 and normal fractional shortening. With Ang II receptor antagonist, LV contractility might be reduced as evidenced by a decrease in (dP/dt)/DP40, probably due to an inhibition of positive inotropic effect of local Ang II in cardiac tissue[6,7]. However, since fractional shortening did not decrease with Ang II receptor antagonist, LV pump function may be preserved due in part to a concomitant reduction of LV wall stress.

In summary, TCV-116 reduced LV contractility with an inhibition of cardiac hypertrophy by blocking the action of local Ang II in cardiac tissue, but LV pump function was preserved due to a concomitant reduction of LV wall stress.

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