Effect of Angiotensin II Receptor Antagonism on Vascular Hypertrophy and Aortic Impedance in Abdominal Aortic-Banded Rat

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Vascular hypertrophy is considered to be an adaptive response to increased arterial wall stress in hypertension. Although there are several reports concerning the effect of angiotensin II inhibition on the development of vascular hypertrophy, little information is available as to its effect on vascular hypertrophy in parallel with the evaluation of arterial wall characteristics. The goal of this study was to evaluate the effect of angiotensin II type 1 receptor antagonist TCV-116 on pressure overload-induced vascular hypertrophy in parallel with the assessment of aortic impedance.

Low dose (LD; 0.3 mg/kg/day) or high dose (HD; 3.0 mg/kg/day) of TCV-116 was administered to abdominal aortic-banded rats over 4 weeks; then hemodynamics and morphology were evaluated. In both the LD and HD groups, blood pressures were decreased to a similar extent compared with those of the vehicle-treated group (P < .05). Left ventricular (LV) weight and LV weight/body weight ratio was inhibited in both TCV-116–treated groups (P < .05), whereas the media cross-sectional area of the aorta was inhibited only in the HD group (P < .05). After the treatment of TCV-116 (LD, HD), total systemic resistance was decreased compared with the vehicle-treated group (P < .05), but there was no significant difference between the TCV-116–treated groups. In contrast, the first harmonic of the impedance modulus revealed the decrease only in the HD group (P < .05).

TCV-116 attenuated the development of pressure overload LV hypertrophy and vascular hypertrophy as well; however, the dose of TCV-116 required for the inhibition of vascular hypertrophy was significantly higher than that for LV hypertrophy. Vascular hypertrophy may be less pressure dependent than cardiac hypertrophy. On chronic addition of high dose of TCV-116, arterial wave reflection was decreased in association with the attenuation of vascular hypertrophy. Am J Hypertens 1999;12:381-387 © 1999 American Journal of Hypertension, Ltd.

Key words: TCV-116, hypertension, angiotensin II receptor antagonist, aortic impedance, rat.
important to assess the effect of inhibition of vascular hypertrophy on the characteristics of the systemic arterial system.

Although there are several reports concerning the effect of angiotensin II inhibition on the development of vascular hypertrophy, little information is available as to its effect on vascular hypertrophy in parallel with the evaluation of arterial wall characteristics. With regard to this, aortic impedance is quite useful for better understanding of arterial wall characteristics because it depends directly on the geometry and mechanical properties of the arterial network and precisely represents LV afterload. Our objective was to evaluate the effect of TCV-116 on pressure overload-induced arterial hypertrophy and on aortic impedance in abdominal aortic-banded rats.

METHODS

Animals and Treatment Male Wistar rats weighing 140 to 170 g, obtained from laboratories (Shizuoka Lab Animal Center, Hamamatsu, Japan) were used for these experiments.

Pressure overload hypertrophy was produced by constriction of the abdominal aorta. The abdominal aorta was surgically isolated above the left renal artery and constricted by using the ligature needle technique as described previously. Sham-operated rats underwent a similar surgical procedure except for the aorta constriction. They were randomly divided into four main groups: sham-operated rats (Sham: n = 6) and three groups of aortic banded rats (vehicle-treated [AC: n = 8], 0.3 mg/kg body weight of TCV-116 [low dose; LD: n = 7], 3.0 mg/kg body weight of TCV-116 [high dose; HD: n = 6]). TCV-116 was suspended in 2% gum arabic solution. The same volume of gum arabic solution was given in vehicle-treated aortic banded rats and vehicle-treated sham control rats. 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 (Osaka, Japan).

Hemodynamic Studies The rats were anesthetized (50 mg/kg thipental sodium intraperitoneally) 24 h after the last administration, then intubated and mechanically ventilated. The right carotid artery was exposed and cannulated with a 2 Fr microtip pressure transducer (model PR249, Millar Instruments, Houston, TX). After right sternal thoracotomy, the ascending aorta was isolated and then the ultrasonic transit time flow probe (T-106, Transonic Systems Inc., Ithaca, NY) was placed for measuring the phasic instantaneous aortic blood flow. The frequency response of the pressure recording channel was flat from 0 to 100 Hz. Pressure was low pass filtered with a corner frequency at 100 Hz. Flow velocity signal was recorded at a 100-Hz filter setting (frequency response -6 dB at 100 Hz). Hemodynamic data were stored on a magnetic tape (Sony Instrumentation Data recorder UN-61430; Sony, Tokyo, Japan) at a tape speed of 9.5 mm/sec. The analog signals were digitized by a 12-bit analog to digital (A/D) converter connected to a microcomputer at intervals of 1 msec and stored on disc. Ten consecutive cardiac cycles were sampled at each stage and averaged.

Aortic input impedance was computed by Fourier analysis of the phasic aortic pressure and flow waves. Characteristic impedance (Zc) was estimated by averaging impedance moduli between 4 and 10 harmonics. The first harmonic of the impedance modulus (Z1) was used as an index of arterial wave reflection. Total systemic resistance (TSR) was determined as input resistance (the modulus of the impedance at zero harmonic).

Changes in daily blood pressure 2 weeks after the chronic administration of TCV-116 also were measured. This study was approved by Animal Care Committee of the School of Medicine, Yamaguchi University.

Histological Studies After the hemodynamic measurements, the heart was arrested by intravenous injection of potassium chloride (2 mEq/mL), and rapidly excised. The right ventricle free wall was trimmed away and the LV weighed.

For morphological analysis of the descending aortic wall, 27 rats (sham n = 6, AC n = 8, LD n = 7, HD n = 6) were fixed with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (PBS), then the aorta was removed. The excised vessel was fixed by buffered formaldehyde before being embedded in paraffin. Sections 4 μm thick were stained with orcein to show the elastic laminae. Media cross-sectional area, defined as the area between the internal and external elastic laminae, was analyzed at × 20 using National Institutes of Health image analysis software.

Statistics Data are expressed as mean ± SD. Individual comparisons among groups were made by analysis of variance (ANOVA) followed by Fisher’s protected least significant difference. A P < .05 was accepted as statistically significant.

RESULTS

As shown in Figure 1, systolic blood pressures were elevated in all three banded groups (AC, LD, and HD) compared with the sham group. After the treatment of TCV-116 (LD, HD), blood pressures were decreased compared with those of the AC group, but no significant difference was observed between the TCV-116-treated groups. On the other hand, pulse pressure was decreased only in the HD group, compared with the
Heart rate did not differ among all groups.

Figure 2 shows the change in daily blood pressure 2 weeks after the chronic administration of TCV-116. Low doses of TCV-116 significantly reduced peak systolic pressure to an extent similar to a high dose of TCV-116 at 8, 16, and 24 h after the last administration of the drug. There was no significant difference in blood pressure between low dose and high dose administration of TCV-116 at any time studied.

Figure 3 represents the effects of TCV-116 on the LV hypertrophy and the aortic hypertrophy. LV weight and LV weight/body weight ratio (LVW/BW) was significantly greater in the AC group than in the sham group. The treatment of TCV-116 inhibited the increase in LV weight and LVW/BW in a dose-dependent manner. There was no difference in body weight among all groups. The aortic media cross-sectional area was also increased in the AC group compared with the sham group; however, only in the HD group was the aortic media cross-sectional area significantly inhibited, even after normalized by cavity area. There was no differences in cavity area among all groups.

The parameters of afterload are summarized in Table 1. The TSR and Z1 were increased in the AC group compared with the sham group. After treatment with TCV-116 (LD, HD), TSR was decreased compared with the AC group, but there was no significant difference between the TCV-116 treated groups (LD and HD). In contrast, Z1 revealed the decrease only in the HD group. Neither cardiac output nor Zc were changed among all groups. The individual impedance spectra for each group are shown in Figure 4. In the HD group, the Z1 decreased and the fluctuation of the impedance moduli was also reduced, indicating less arterial wave reflection than in other banded groups.

DISCUSSION

The major findings of this study are as follows. First, TCV-116 attenuated the development of pressure-overload LV hypertrophy and vascular hypertrophy as well; however, the dose of TCV-116 required for the inhibition of vascular hypertrophy was significantly higher compared with that for LV hypertrophy. Second, on chronic addition of a high dose of TCV-116, arterial wave reflection was decreased in association with the attenuation of vascular hypertrophy.

Vascular Hypertrophy and Angiotensin II Vascular hypertrophy expressed as the media thickening, involving smooth muscle cell and extracellular matrix
FIGURE 3. Bar graphs showing the effect of TCV-116 on the development of left ventricular hypertrophy and aortic hypertrophy in abdominal aortic banded rats. Data are expressed as means ± SD. Open bars, sham-operated rats (Sham); solid bars, vehicle-treated rats with aortic constriction (AC); hatched bars, rats with aortic constriction and 0.3 mg/kg/day of TCV-116 (LD); cross-hatched bars, rats with aortic constriction and 3.0 mg/kg/day (HD). *P < .05 v Sham; †P < .05 v AC; ‡P < .05 v LD.

components, is widely considered to be an adaptation to increased arterial wall stress. Although it is accepted that the mechanical stress from an increase in afterload stimulates vascular hypertrophy, recent evidence suggests that humoral factors may also play an important role in the development of vascular hypertrophy. Particularly, angiotensin II can stimulate vascular smooth muscle cell growth in vitro and influence extracellular matrix. A direct contribution of angiotensin II to vascular hypertrophy has been suggested by the findings that in vivo systemic infusion of angiotensin II with administration of hydralazine induced vascular hypertrophy without a pressor response and that AT1 receptor antagonist induced a prevention of vascular hypertrophy independent from a change in blood pressure. Morishita et al provided the first evidence that overexpression of an autocrine/paracrine factor (ie, angiotensin) transfected into the intact vessel wall in vivo mediates the vascular remodeling process of hypertension independent of systemic factors or hemodynamic stimuli. Consistent with these reports, we observed the inhibition of pressure over-

TABLE 1. AFTERLOAD PARAMETERS 4 WEEKS AFTER AORTIC CONstriction

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 6)</th>
<th>AC  (n = 8)</th>
<th>LD  (n = 7)</th>
<th>HD  (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Cardiac output (mL/min)</td>
<td>24 ± 5</td>
<td>21 ± 4</td>
<td>22 ± 4</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>TSR (10^5 dynes · sec/cm^5)</td>
<td>382 ± 44</td>
<td>536 ± 94*</td>
<td>424 ± 94t</td>
<td>415 ± 102t</td>
</tr>
<tr>
<td>Z1 (10^5 dynes · sec/cm^5)</td>
<td>26.4 ± 4.3</td>
<td>38.6 ± 4.4*</td>
<td>33.1 ± 12.7</td>
<td>27.8 ± 9.2t</td>
</tr>
<tr>
<td>Zc (10^5 dynes · sec/cm^5)</td>
<td>15.5 ± 5.0</td>
<td>15.5 ± 3.9</td>
<td>21.3 ± 9.4</td>
<td>19.3 ± 11.4</td>
</tr>
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Values are expressed as means ± SD.

TSR, total systemic resistance; Z1, impedance modulus at first harmonic; Zc, characteristic impedance; Sham, sham-operated rats; AC, vehicle-treated rats with aortic constriction; LD, rats with aortic constriction and 0.3 mg/kg/day of TCV-116; HD, rats with aortic constriction and 3.0 mg/kg per day of TCV-116.

*P < .05 v Sham.
†P < .05 v AC.
load–induced vascular hypertrophy by AT1 receptor antagonist TCV-116. Furthermore, we demonstrated that the dose of TCV-116 required for the inhibition of hypertrophy was different between LV and aorta.

Namely, a low dose of TCV-116 significantly inhibited LV hypertrophy with a reduction in blood pressure, whereas a high dose was required for an inhibition of vascular hypertrophy without further reduction in blood pressure. Consistent with our findings, Ledingham and Laverty\textsuperscript{7} reported that the lowest dose (0.3 mg/kg/day) of angiotensin II receptor antagonist valsartan inhibited the increase in LV mass associated with a reduction in blood pressure, whereas it had no effect on the media volume of mesenteric artery in New Zealand genetically hypertensive rats. In contrast, Shaw et al\textsuperscript{1\textsuperscript{8}} found that, in SHR, the lowest dose (1 mg/kg) of the angiotensin II antagonist D8731 reduced the media/lumen ratio of the mesenteric vessels to close to that of the WKY rats, but did not change LV weight. However, in the latter study, the lowest

FIGURE 4. Individual aortic input impedance spectra in all groups. The values of total systemic resistance (the modulus at zero harmonic) were indicated within each graph. In the HD group, the Zl decreased and the fluctuation of the impedance moduli was also reduced, indicating less arterial wave reflection than in other banded groups.
dose of D8731 induced a substantial reduction in blood pressure only in 20-week SHR and not obviously in 4 to 16 week SHRs. The lesser pressure reduction may lead to a lesser inhibitory effect of D8731 on LV hypertrophy. In both studies, a high dose of these drugs, by which blood pressure was significantly decreased, induced a significant attenuation of either LV or vascular hypertrophy. Taken together, the inhibition of vascular hypertrophy by angiotensin II receptor antagonists may be less pressure-dependent than LV hypertrophy, and also the affinity to the angiotensin II receptor may be different among various angiotensin II receptor antagonists. With regard to this, Raasch et al.\(^\text{19}\) compared the effects of ACE inhibitors (captopril, enalapril, fosinopril, and ramipril) with those of the AT1-receptor antagonist HR 720 with respect to their ability to induce regression of hypertrophy of LV and aorta in SHRs. In their studies, both ACE inhibitors and AT1 receptor antagonist reduced the thickness of vascular media, independent of the reduction in blood pressure. However, for regression of LV hypertrophy, the pressure reduction was inevitable.

**Effect of TCV-116 on Aortic Impedance** Aortic input impedance, which represents LV afterload, is considered a major determinant for the development of pressure overload LV hypertrophy and vascular hypertrophy as well.\(^\text{6,20,21}\) Because TCV 116 provides an inhibition of vascular hypertrophy, probably either by reducing the vascular wall stress or by a direct inhibition of tissue angiotensin II, it is important to assess the change in aortic input impedance after the addition of TCV-116. To our knowledge, there is no report of evaluating the effect of angiotensin II inhibition either by an ACE inhibitor or an AT1 receptor antagonist on aortic input impedance, with regard to the inhibition or prevention of vascular hypertrophy. The most important aspect of this study is that there was a different effect of TCV-116 on aortic impedance depending on the dose used: namely, systemic vascular resistance was easily decreased by a low dose of TCV-116, probably through the mechanism of functional vasodilation, whereas a high dose was required to reduce the arterial wave reflection. The decrease in pulse pressure observed in the high dose TCV-116 group was mostly caused by the reduction in the late systolic rise of aortic pressure induced by augmented arterial wave reflection with aortic banding.

Because the magnitude of arterial wave reflection is substantially influenced by the stiffness of small to medium sized arteries,\(^\text{22}\) the observed inhibition of vascular hypertrophy by a high dose of TCV-116 might be involved in the mechanism by which arterial wave reflection decreased. These inhibitory effects of angiotensin II receptor antagonists on vascular hypertrophy may contribute to the reduction of pressure overload-induced LV hypertrophy.

**REFERENCES**


