Original Article

Long-Term Treatment with Valsartan Improved Cyclic Variation of the Myocardial Integral Backscatter Signal and Diastolic Dysfunction in Hypertensive Patients: The Echocardiographic Assessment

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Myocardial fibrosis is the major determinant of diastolic property of the left ventricle (LV). Experimental and clinical studies have suggested that angiotensin receptor blockers attenuate myocardial fibrosis in various heart diseases. The integrated backscatter signal (IBS) represents a promising ultrasonic method for assessing the characterization of myocardial tissue: cardiac cycle–dependent variation of the IBS (IBS-CV) is negatively correlated with myocardial collagen deposition in hypertensive hearts. Using non-invasive echocardiographic techniques, we performed a prospective, multi-center trial to examine whether long-term treatment with valsartan would improve myocardial fibrosis and diastolic dysfunction in hypertensives. This study included 43 hypertensive patients who had impaired diastolic function (transmitral Doppler flow early to late filling velocity ratio [*E*/*A* ratio] <1.0) and preserved systolic function (LV ejection fraction [LVEF] >50%). Twelve-month valsartan treatment reduced blood pressure (BP) and LV mass index. Valsartan significantly increased not only IBS-CV but also *E*/*A* ratio without changing LVEF. The effects of valsartan were compared between two subgroups: one with low IBS-CV (IBS-CV <5.08 dB [the average of 43 patients at baseline]), the other with high IBS-CV (IBS-CV >5.08 dB). At baseline, BP, LV mass index, LVEF, and *E*/*A* ratio were similar in the two groups. Valsartan significantly increased IBS-CV and *E*/*A* ratio in the low IBS-CV group, but not in the high IBS-CV group, despite comparable reductions in BP and LV mass. In conclusion, long-term valsartan treatment attenuated myocardial fibrosis and improved diastolic dysfunction in hypertensives. It is suggested that in the low IBS-CV group, improvement of diastolic dysfunction by valsartan may be caused by attenuation of myocardial fibrosis, and not by regression of LV hypertrophy. (*Hypertens Res* 2008; 31: 1835–1842)

***Key Words*: hypertension, angiotensin receptor blocker, myocardial fibrosis, diastolic function, cardiac hypertrophy**

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Introduction

As many as 50% of patients diagnosed as having congestive heart failure show preserved systolic function but impaired diastolic function of the left ventricle (LV). Hypertension is a major cause of diastolic dysfunction (*1*, *2*). Excessive myocardial fibrosis and LV hypertrophy in hypertensives are the major determinants of diastolic dysfunction, which is characterized by decreased diastolic distensibility and impaired relaxation (*2–5*). There are no specific treatments to improve diastolic dysfunction at present.

Recently, angiotensin receptor blockers (ARBs) have been highlighted as possible candidates for the treatment of diastolic dysfunction in hypertensive hearts. An anti-fibrotic effect of ARBs on hypertensive heart has been established in animal models (*6–8*). In hypertensive patients, 12-month treatment with losartan, but not amlodipine, reduced myocardial collagen content in hypertrophied hearts, as demonstrated by endomyocardial biopsy histology (*9*). Another clinical study demonstrated that losartan reduced myocardial collagen fraction and improved LV stiffness only in hypertensive patients who had endomyocardial biopsy-proven massive fibrosis and severe diastolic dysfunction (*10*). These studies provided evidence of anti-fibrotic effects of ARBs in humans using invasive methods. Although losartan and irbesartan have been shown to decrease circulating levels of markers of tissue collagen synthesis (*e.g.,* the C-terminal peptide of procollagen type I) in hypertensive patients (*11*, *12*), the serological markers are not exclusively heart-specific.

Accordingly, the aim of this study was to use non-invasive echocardiography to investigate whether ARB treatment would regress myocardial fibrosis and diastolic dysfunction in hypertensive patients. The integrated backscatter signal (IBS) is a non-invasive measure of the acoustic characterization of myocardial tissue. It has been shown that reduction in the cardiac cycle–dependent variation of the IBS (IBS-CV) reflects myocardial collagen deposition in hypertensive hearts (*13*, *14*). Thus, we performed a prospective multi-center trial to determine the effects of 12 months of treatment with valsartan on myocardial IBS-CV, LV hypertrophy, and LV function as assessed by echocardiography.

Methods

The design of this study was a 12-month prospective, multicenter trial performed by Valsartan Diastolic Function Improvement Study investigators. The study protocol was approved by the Ethics Committee for the Clinical Research of Kurume University for Medical Sciences. All the enrolled patients gave written informed consent.

Study Population and Protocol

Outpatients with a history of treated or untreated hypertension were enrolled from April 1, 2004 to March 31, 2005, when the patients fulfilled the inclusion criteria: 1) systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg, 2) normal sinus rhythm, 3) LV ejection fraction $>50\%$ by echocardiography (Teichholtz's method) and 4) E/A ratio <1.0 on transmitral Doppler spectra. The exclusion criteria were administration of medication of ARB or angiotensin-converting enzyme inhibitor (ACEI), symptoms or signs of congestive heart failure within the past year, and the presence of renal failure (serum creatinine >2 mg/dL) or malignancies.

At baseline, medical history, smoking habits, and medications were evaluated. Sphyngmomanometric blood pressure measurement was performed after 5 min rest in the sitting position, and at least two measurements were averaged. All patients underwent standard/Doppler echocardiographic examination and myocardial IBS analysis.

The starting dose of valsartan was 40 or 80 mg/d. Blood pressure measurement was repeated on each monthly visit to the outpatient clinic. The dose of valsartan was titrated to achieve a target blood pressure (140/90 mmHg or lower). The maximal dose of valsartan was 160 mg/d, the approved maximum dose in Japan. A calcium channel blocker, α-blocker or diuretic was added if the target was not reached by the maximum dose of valsartan. Addition of an ACEI, β-blocker, or other type of ARB was not permitted. Standard/Doppler echocardiography and IBS analysis were repeated after the 12 months of treatment.

Echocardiographic Data Acquisition

A SONOS ultrasound system with IBS analysis software (Philips, Tokyo, Japan) was used for two-dimensional, targeted M-mode and Doppler ultrasound recordings followed by backscatter tissue characterization. Standard echocardiographic views, including parasternal long-axis and apical four- and two-chamber views, were obtained in two-dimensional and Doppler flow modes. For IBS analysis, a region of interest was placed at the middle segment of the septum in the parasternal short-axis view (*11*). The same myocardial area could be analyzed in each patient at baseline and post-treatment studies by the use of anatomical landmarks on the image. In the region, backscatter images were acquired in continuous loop review format. The echocardiographic and IBS images were stored on a diskette in digital format.

Echocardiographic Data Analysis

The echocardiographic and IBS data were collected and analyzed in the core laboratory (Kurume University). Interventricular septal thickness and posterior wall thicknesses at end-diastole (mm) and LV end-diastolic and end-systolic dimensions (mm) were determined from M-mode echocardiogram. LV ejection fraction (%) was calculated using Teichholtz's formula. Estimated LV mass was calculated with Devereux's

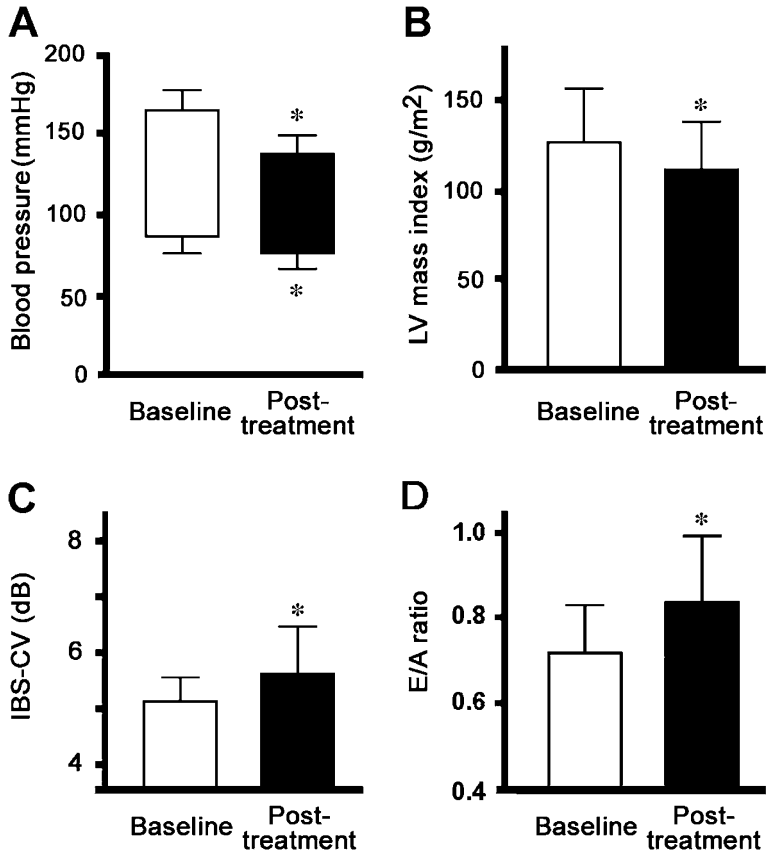

Fig. 1. *Effects of 12-month treatment with valsartan on blood pressure (A) (top, systolic blood pressure; bottom, diastolic blood pressure), LV mass index (B), the cardiac cyclic–dependent variation of myocardial integrated backscatter intensity (IBS-CV)(C), and early to late filling velocity ratio of transmitral pulse-wave Doppler spectra (E/A ratio)(D) in all patients. *$p < 0.01$ vs. baseline. Bar = 1 ×SD.*

formula, and LV mass index (g/m^2) was determined by dividing the LV volume by body surface area (*15*). Pulse-wave Doppler transmitral flow velocity was obtained by positioning a sample volume at the mitral tip level in an apical four-chamber view. An early to late filling velocity ratio of transmitral pulse-wave Doppler spectra (*E*/*A* ratio) was calculated. The average of three independent beats was used for analysis of each parameter. Data were analyzed by three expert echocardiographers in a blind manner and data from these cardiographers were averaged.

For determining myocardial IBS value and the IBS-CV, a time-dependent curve of the backscatter signal was obtained. The highest value (end-diastolic frame) and the lowest value (end-systolic frame) of the IBS were selected, and the difference between the diastolic and systolic values was defined as the IBS-CV. In each patient, the average of three independent beats was used for analysis. The signal analysis was performed in a blind fashion by three echocardiographers who were unaware of the conventional and Doppler echocardiographic data. The results of the three echocardiographers were averaged.

Subgroup Analysis

Effects of valsartan were compared between two subgroups: the low and high IBS-CV groups. The low IBS-CV group included patients whose IBS-CV was below the average of the IBS-CV at baseline. The high IBS-CV group had an IBS-CV above the average of the IBS-CV at baseline.

Statistical Analysis

Data are expressed as the mean±SD unless stated otherwise. Comparison between baseline and post-treatment studies was evaluated by paired Student's *t*-test. In subgroup analysis, unpaired Student's *t*-test and repeated measure analysis of variance followed by Dunn's multiple comparison procedure were performed to compare the baseline characteristics and effects of treatment between the two subgroups, respectively. A value of $p < 0.05$ was considered statistically significant. Intra- and interobserver variations of echocardiographic and IBS measurements were <5%.

Table 1. Demographic Data at Baseline

	High IBS-CV	Low IBS-CV
Male/female (*n*)	7/13	10/13
Age (years)	64±12	63±14
Heart rate (/min)	69±6	66±10
Systolic blood pressure (mmHg)	164±12	166±19
Diastolic blood pressure (mmHg)	86±13	92±13
LV mass index (g/m^2)	122±29	128±34
LV ejection fraction (%)	71±5	70±6
Serum creatinine (mg/dL)	0.73±0.16	0.79±0.24
Estimated creatinine clearance (mL/min)	70.5±17.9	66.2±13.5
Coexisting disease (*n*)		
History of heart failure	0	1
Diabetes mellitus	6	5
Dyslipidemia	8	9
Angina pectoris	2	3
Old myocardial infarction	0	0

Data are the mean±SD. Unpaired *t*-test or χ^2 test did not reveal statistically significant differences in any parameters between the two groups. Estimated creatinine clearance was computed with the Cockcroft-Gault equation (*31*). Diabetes mellitus was defined by the presence of fasting plasma glucose ≥126 mg/dL and/or hemoglobin A1c ≥6.4% or medication by oral antidiabetics. Dyslipidemia was diagnosed when low-density lipoprotein cholesterol was ≥140mg/dL, high-density lipoprotein cholesterol <40 mg/dL, and/or triglycerides ≥150 mg/dL. IBS-CV, cardiac cycle–dependent variation of the integrated backscatter signal; LV, left ventricular.

Results

This study enrolled 46 consecutive patients. Of the enrolled patients, three were excluded from the analysis because the quality of their IBS recording was not high enough to enable evaluation at baseline. Accordingly, a total of 43 patients (63±13 years old, 17 men) were analyzed in this study.

Effects of 12-Month Treatment with Valsartan

The starting dose of valsartan was 62±20 mg/d, and the maintenance dose was 82±33 mg/d. The12-month treatment with valsartan significantly decreased SBP and DBP and reduced LV mass index (Fig. 1A, B). At baseline, the average of myocardial IBS-CV was 5.08±0.51 dB. Valsartan significantly increased the IBS-CV after 12 months of treatment (Fig. 1C). Moreover, the *E*/*A* ratio was significantly increased (Fig. 1D), whereas LV ejection fraction was not affected by the treatment (data not shown).

Subgroup Analysis

Next, we compared the effects of valsartan between two subgroups: the low and high IBS-CV groups. The low IBS-CV group ($n=23$) included patients who had IBS-CV <5.08 at baseline (the average of the total patients). The high IBS-CV group consisted of 20 patients with IBS-CV ≥5.08 at baseline. At baseline, age, heart rate, SBP, DBP, LV mass index, and renal function did not differ between the high and low IBS-CV groups (Table 1). In addition, there were no differences in the prevalence of male gender, coexisting diseases, or previous antihypertensive treatment (Tables 1 and 2).

The starting and maintenance doses of valsartan were similar in the high and low IBS-CV groups (Table 2). The two groups were also comparable in the prevalence of other antihypertensive agents after treatment. The12-month valsartan treatment significantly decreased SBP and DBP in the high and low IBS-CV groups to similar levels (Fig. 2A). LV mass index in the two groups was also reduced similarly by valsartan treatment (Fig. 2B). Heart rate was not changed by valsartan in either group (Fig. 2C).

In the low IBS-CV group, the IBS-CV level was significantly increased by valsartan to a level comparable to that in the high IBS-CV group, whereas the increase in the IBS-CV was not significant in the high IBS-CV group (Fig. 3A). As shown in Fig. 3B, *E*/*A* ratio did not differ between the low and high IBS-CV groups at baseline. Valsartan significantly improved *E*/*A* ratio in the low IBS-CV group, but not in the high IBS-CV group. LV ejection fraction was not affected by valsartan in either group (data not shown).

Discussion

The present study has demonstrated for the first time that long-term treatment with valsartan increases myocardial IBS-CV and transmitral Doppler spectra *E*/*A* ratio in hypertensive patients with preserved systolic but impaired diastolic LV function. The beneficial effects of valsartan were significant in the subgroup with low baseline IBS-CV but not in the subgroup with high IBS-CV, whereas blood pressure and LV mass were decreased similarly in both groups. Thus, it is suggested that in the low IBS-CV group, improvement of diastolic dysfunction by valsartan may be caused by attenuation of myocardial fibrosis and not by regression of LV hypertrophy.

Ultrasonic IBS reflects the acoustic properties of the myocardium, particularly structural changes of the fibrous component, in both normal subjects and patients with various heart diseases (*16–21*). Among the tissue characterization parameters, the IBS-CV and the peak diastolic IBS value are commonly used as non-invasive parameters of myocardial fibrosis in hypertensive patients. However, the IBS-CV analysis has higher sensitivity than the peak diastolic IBS analysis (*13*, *14*, *22*). Thus, in the present study, the IBS-CV was used as a measure of myocardial fibrosis. Moreover, we chose a

Table 2. Medication

	High IBS-CV		Low IBS-CV	
	Baseline	Post- treatment	Baseline	Post- treatment
Valsartan dose (mg/d)	62±20	80±26*	63±21	83±21*
Other antihypertensive medication (*n*)				
Any preceding antihypertensive agents	13	—	13	—
Calcium channel blocker	12	13	9	12
β-Blocker	1	1	4	4
α-Blocker	1	2	1	1
Diuretics	2	3	1	3

*$p<0.05$ *vs*. baseline. Unpaired *t*-test or χ^2 test did not reveal a statistically significant difference in the prevalence of antihypertensive agents between the two groups.

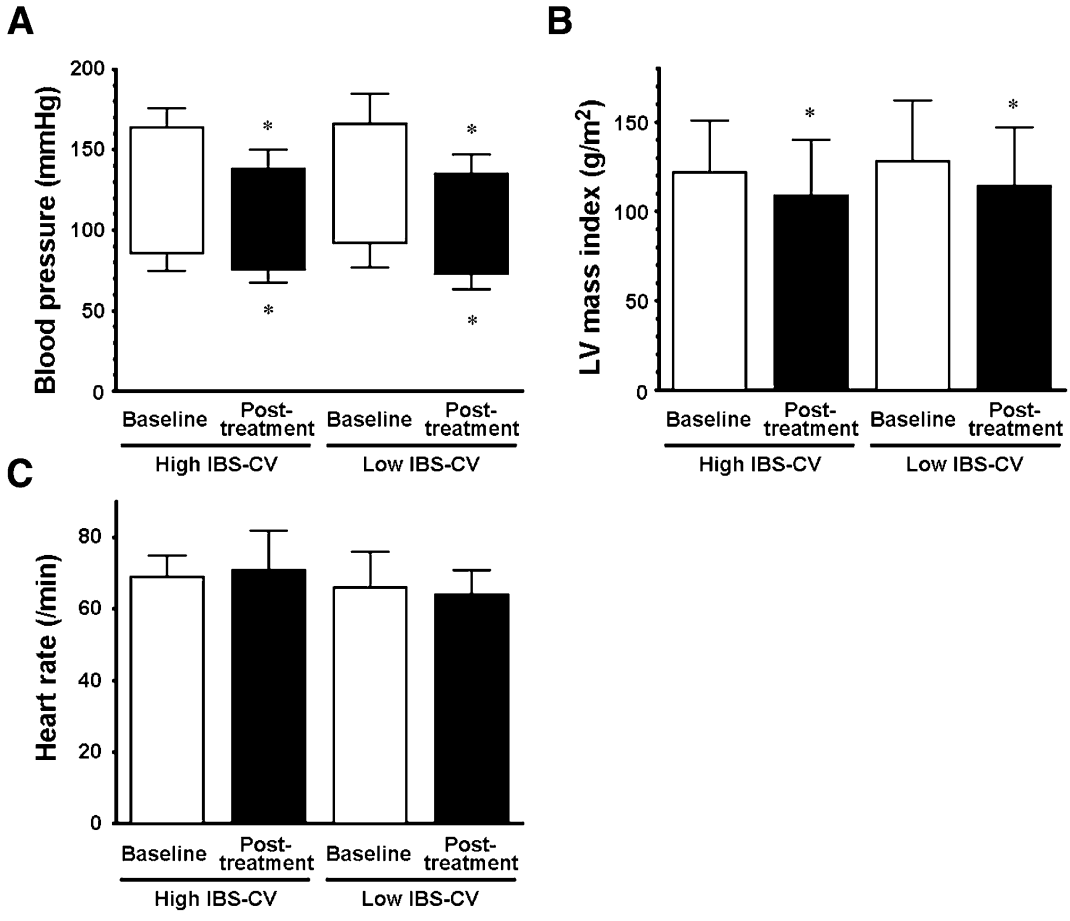

Fig. 2. *Comparison of the effects of 12-month treatment with valsartan between the high and low IBS-CV groups. A: Blood pressure (top, systolic blood pressure; bottom, diastolic blood pressure). B: LV mass index. C: Heart rate. *p < 0.01 vs. baseline. Bar = 1 × SD.*

treatment period long enough to reduce a significant amount of collagen in the myocardium, based on the half-life of fibrillar collagen (about 6 months) (*23*).

The present study enrolled hypertensive patients who had preserved systolic function and modestly impaired diastolic dysfunction, which was characterized by an impaired relaxation pattern of transmitral Doppler spectra. Most patients had mild to moderate LV hypertrophy with LV mass indexes ranging from 96 to 166 g/m^2 (10–90 percentile). Previous studies reported that the IBS-CV of the mid-septum in normo-

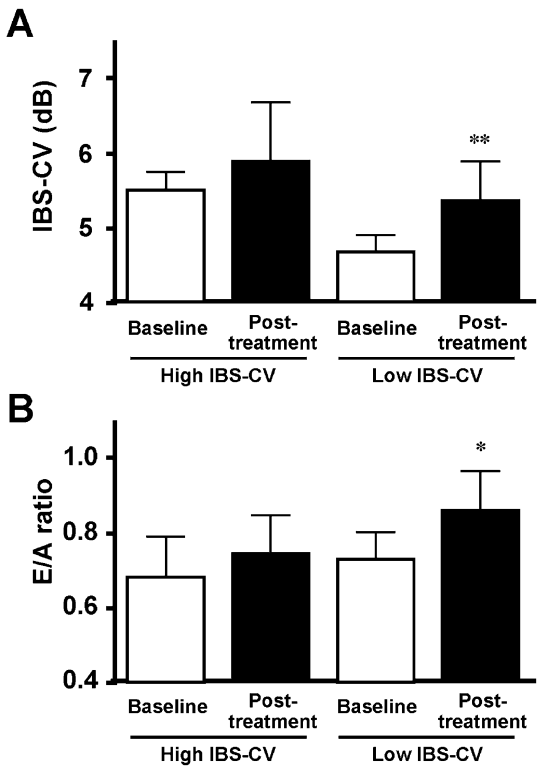

Fig. 3. *Effects of 12-month treatment with valsartan on the IBS-CV (A) and E/A ratio (B) in the high and low IBS-CV groups. *$p<0.05$ and **$p<0.01$ vs. baseline, respectively. Bar = 1 ×SD.*

tensive subjects was 6.5–7.0 dB (*13*, *14*), which was higher than the IBS-CV levels (5.08±0.51 dB) in our patients. Thus, it is suggested that myocardial collagen deposition was increased in patients in this study.

The most important finding of this study is that valsartan significantly increased the IBS-CV and *E*/*A* ratio (Fig. 1C, D), suggesting attenuation of myocardial fibrosis and diastolic dysfunction. The valsartan-induced reduction in myocardial fibrosis is in accord with previous studies using biopsied tissues (*9*, *10*). In this study, valsartan decreased blood pressure below the target levels (<140/90 mmHg) and regressed LV hypertrophy (Fig. 1A, B). Thus, it remained unknown whether reduction of myocardial fibrosis, blood pressure lowering, LV mass regression, or a combination of these effects contributed to the improvement of *E*/*A* ratio by valsartan.

Because a histological study demonstrated that losartan reduced myocardial collagen content only in hypertensive patients with severe myocardial fibrosis (*10*), we compared the effects of valsartan between the low IBS-CV group (more fibrotic) and the high IBS-CV group (less fibrotic). As expected, valsartan significantly improved the IBS-CV and *E*/*A* ratio only in the low IBS-CV group (Fig. 3). Because both groups showed similar reductions in blood pressure and LV mass (Fig. 2), the differences in the effects on the IBS-CV and *E*/*A* ratio were independent of the valsartan-mediated reduction of blood pressure and LV hypertrophy. Heart rate was not affected by valsartan in either group. Moreover, the dose of valsartan and the prevalence of other antihypertensive agents were not different between the two subgroups. None of the enrolled patients was taking aldosterone antagonists, which may reduce myocardial fibrosis (*24*). Because β-blockers may affect *E*/*A* ratio in hypertensive patients (*25*), a subgroup analysis was performed by excluding five patients treated with a β-blocker, but the results were not different without those patients (data not shown). Accordingly, it is suggested that improvement of diastolic function may be caused by valsartan-induced attenuation of myocardial fibrosis in the low IBS-CV group. Collagen turnover is enhanced in hypertensive heart with severe myocardial fibrosis (*26*, *27*). Thus, it is possible that patients with enhanced collage turnover might be more susceptible to the anti-fibrotic action of ARBs than patients with less fibrosis. This study did not clarify the determinant factors of diastolic dysfunction in the high IVS-CV group.

IBS-CV can be affected by many factors, such as fibrosis, anisotropy, and the contractile performance of the myocardium (*19*, *28*, *29*). Anisotropy is the artifactual IBS change influenced by the angle between the ultrasonic beam and the myocardial fiber. In this study, anisotropy was avoided as much as possible by positioning the region of interest in the mid-septum in the parasternal short-axis view because the myocardial fiber of the segment is oriented almost perpendicular to the ultrasound beam. In hypertrophied hearts, the intrinsic myocardial contractility may be impaired preceding apparent reduction in LV ejection fraction (*30*). Thus, it is possible that the increases in the IBS-CV in this study may be partially due to reversal of intrinsic myocardial contractility by valsartan treatment. Also, infarcted myocardium is known to show reduced IBS-CV (*17*, *19*). The present study did not include patients having a history of myocardial infarction. Because myocardial ischemia may have effects on IBS-CV, we performed a subgroup analysis by excluding patients with angina pectoris, but the results were not different without those patients (data not shown).

Limitations of This Study

The lack of a control group who did not have ARB limits our interpretation and discussion in this study. Moreover, because none of the patients had clinical indications of endomyocardial biopsy, we could not confirm the correlation between IBS-CV and severity of histological fibrosis. In addition, duration of hypertension and diurnal blood pressure difference may affect myocardial fibrosis and the effects of valsartan. Finally, this study enrolled only patients having modest diastolic dysfunction characterized by delayed relaxation pattern; a future study should address the effects of valsartan in hypertensive patients with advanced diastolic dysfunction.

In conclusion, the present study using echocardiographic techniques has provided evidence that long-term valsartan treatment attenuates myocardial fibrosis and improves diastolic dysfunction in hypertensive patients. Thus, ARBs may be desirable for preventing diastolic heart failure in hypertensive patients.

Appendix

Valsartan Diastolic Function Improvement Study investigators were the following: Tsutomu Imaizumi, Hisashi Kai, Yoshihiko Mizuta (Kurume University Hospital); Takahiro Matsumoto, Koji Hiyamuta (National Hospital Organization Kyushu Medical Center); Takafumi Ueno (Fukuoka City Medical Association Hospital); Hideki Yoshiyama (Yame General Hospital); and Tatsuro Hiraki (Omuta City General Hospital).

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