Original Article

Long-Term Anti-Hypertensive Therapy with Benidipine Improves Arterial Stiffness over Blood Pressure Lowering

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Pulse wave velocity (PWV) reflects arterial stiffness and is an independent predictor of cardiovascular mortality and morbidity. However, because it is closely related to blood pressure (BP), PWV is an imperfect measure for evaluating the effects of anti-hypertensive drugs on arterial wall properties. To clarify the effect of benidipine on arterial properties, we first derived the regression line between BP and PWV changes in a short-term experiment. Using this line, we evaluated the long-term effect of benidipine on PWV changes. In the short-term experiment, 29 participants were intravenously administered nicardipine for 90 min. Maximum decreases of brachial-ankle PWV (baPWV) were plotted against the corresponding decreases in BP. In the long-term experiment, 9 hypertensive patients were treated with benidipine for 1 year, during which BP and baPWV were monitored. After 1 year, benidipine was suspended for 2 weeks, and BP and baPWV were reevaluated. In the short-term experiment, PWV was dependent on BP only, and the equation of the regression line was $\Delta\text{PWV (cm/s)} = 10.114 \times \Delta\text{MBP (mmHg)}$ ($r=0.913$) or $\Delta\text{PWV (\%)} = 0.719 \times \Delta\text{MBP (\%)}$ ($r=0.926$). In the long-term therapy, benidipine treatment achieved stable BP control within 3 months; the real PWV decreases (r-PWV) were almost identical to the PWV decrease estimated (e-PWV) from BP lowering at 3 months. However, r-PWV exceeded e-PWV after 6 months. Relative BP and PWV improvements compared to the control were maintained 2 weeks after suspension of benidipine. In conclusion, long-term benidipine administration improves arterial wall properties beyond what can be accounted for by changes in BP. (*Hypertens Res* 2005; 28: 959–964)

Key Words: pulse wave velocity, blood pressure, benidipine, nicardipine, human

Introduction

Arterial stiffness has been recognized as an important indicator of increased cardiovascular risk. Several studies have clearly demonstrated that increased arterial stiffness, as determined by pulse wave velocity (PWV), is a strong risk factor for cardiovascular morbidity and mortality (1–3). PWV mea-

surement has been rapidly deployed in medical practice since a convenient automated pulse wave analyzer became available commercially in Japan. Brachial-ankle PWV (baPWV) derived using the automated analyzer is also closely related to risk factors and/or the organ damage characteristic of cardiovascular diseases (4–9). Moreover, significant improvements in baPWV have been observed after anti-hypertensive treatments with different classes of drugs (10–12), and thus

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Fig. 1. *A:* The correlation between maximum changes of mean blood pressure (MBP) and concomitant changes of pulse wave velocity (PWV) after nicardipine administration. *B:* The correlation between the changes of MBP and PWV in all-data analysis.

Table 1. Time-Dependent Effects of Benidipine on the Indicated Parameters

	Control	Treatment with benidipine			2 week after suspension
		6 months	12 months	<i>p</i> in trend	
SBP (mmHg)	158.8±3.6	127.1±3.3**	128.8±3.0**	<0.0001	146.0±4.5 ^{##}
DBP (mmHg)	96.7±4.0	80.6±3.1**	79.9±3.2**	0.0012	87.7±4.0 ^{##}
MBP (mmHg)	117.4±3.4	96.1±2.9**	96.2±2.2**	<0.0001	107.1±4.4 ^{##}
HR (bpm) [†]	69.1±2.7	69.3±3.2	69.1±3.4	1.00	63.8±1.6
PWV (cm/s)	1,768±100	1,464±59*	1,449±69**	0.012	1,631±77 [#]
CRP (ng/ml)	1,163±279	651±108	520±101*	0.05	716±232
t-AM (fmol/ml)	12.8±0.8	12.2±0.3	12.5±0.4	0.78	14.0±0.7
m-AM (fmol/ml)	1.68±0.16	1.66±0.15	1.59±0.10	0.90	1.75±0.23
ET-1 (pg/ml)	2.04±0.16	1.79±0.19	1.84±0.17	0.57	1.56±0.11
PRA (ng/ml/h)	1.06±0.20	1.84±0.23	1.69±0.35	0.11	0.86±0.14
PAC (pg/ml)	92.7±9.1	104.8±9.8	100.4±10.6	0.68	103.4±14.0
IRI (μU/ml)	13.7±6.1	11.9±6.0	8.8±1.8	0.79	9.4±3.3

SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; PWV, pulse wave velocity; CRP, C-reactive protein; t-AM, total adrenomedullin; m-AM, mature adrenomedullin; ET-1, endothelin-1; PRA, plasma renin activity; PAC, plasma aldosterone concentration; IRI, immunoreactive insulin. *p<0.05, **p<0.01, compared to control (ANOVA); [#]*p*<0.05, ^{##}*p*<0.01, compared to control (paired *t*-test). [†]HR was measured by ECG.

baPWV may be a surrogate marker of the efficacy of anti-hypertensive therapy. However, baPWV, like authentic PWV, is itself closely dependent on blood pressure (BP); thus it has been difficult to evaluate whether improvements in baPWV with anti-hypertensive therapy really reflect an improvement in arterial properties.

Ca²⁺ channel blockers (CAB) have been reported to possess anti-sclerotic properties, and CAB have improved cervical artery and coronary artery sclerosis in randomized controlled trials (13–15). Benidipine has additional beneficial actions on endothelial function and inhibits intimal thickening in animal studies (16–18); thus benidipine could be a more effective treatment for arterial sclerosis in hypertensive patients. To clarify the effect of benidipine on arterial properties, we first

derived the regression line between BP and baPWV changes in a short-term experiment using nicardipine. We then used this regression line to evaluate the long-term effect of benidipine on baPWV changes separately from BP changes in hypertensive patients.

Methods

Short-Term Experiment

Participants

Twenty-nine male participants, mean age 46.0±9.2 years, were enrolled in this study. All participants received screening examinations including urine and blood tests, ECG, chest

Fig. 2. Comparison between real pulse wave velocity (PWV) changes (closed column) and estimated PWV changes (open column: analysis with maximum changes; gray column: all-data analysis) after benidipine treatment in hypertensive patients (mean $\pm$ SEM, $n=9$). Estimated PWV changes were calculated from blood pressure changes as described in the text.

X-ray, and echocardiography. They were diagnosed as normotensive ($n=11$), hypertensive ($n=11$), or diabetic ($n=8$), and one patient had both hypertension and diabetes mellitus. They were not receiving any medication, nor was there any evidence of heart failure (left ventricular ejection fraction $<50\%$), renal failure (serum creatinine >1.0 mg/dl), peripheral artery disease, or a history of cardiovascular accidents. The study was approved by the ethics committee of our institute, and all participants gave written informed consent.

Study Protocol

All experiments were started at 9 AM in the fasting state. One 20-gauge cannula was inserted into the forearm vein for infusion of 0.9% saline. Saline was infused at a rate of 100 ml/h throughout the experiments. Baseline measurements were obtained after a 60 min equilibration period. Nicardipine ($1 \mu\text{g}/\text{min}/\text{kg}$) was then administered intravenously for 90 min, followed by a 90 min saline only infusion. After 45 min, the dosage of nicardipine was increased to $1.5 \mu\text{g}/\text{min}/\text{kg}$ to generate a 10% reduction of systolic BP (SBP), but was not increased above $1.5 \mu\text{g}/\text{min}/\text{kg}$ for safety reasons, even when a small BP reduction occurred. For convenience, BP was monitored every 10 min using an automated hemodynamometer with a brachial cuff. At 15 min intervals, PWV, BP and heart rate were measured using an automatic waveform analyzer (form PWV/ABI, BP-203RPE; Colin Medical Technology Co., Komaki, Japan) as reported previously (4).

Maximum reductions in PWV were observed at 60–90 min after nicardipine infusion. Maximum reductions in PWV and concomitant BP reductions were plotted (linear regression), and the regression formula obtained. All data obtained during nicardipine infusion (5 points per person) were also analyzed by multivariate regression analysis.

Long-Term Treatment

Patients

Twelve patients with untreated essential hypertension were enrolled in this study. However, two patients dropped out within 3 months and one patient had uncontrollable hypertension. The remaining nine patients (53.0 ± 13.3 years of age, 6 men and 3 women) completed the study and were used for the analysis. All patients received screening examinations, including urine and blood tests, ECG, chest X-ray, and echocardiography. No patient was receiving medication for hypertension, and no patient had heart failure, renal failure, peripheral artery disease, or a history of cardiovascular accidents. Three patients had hypercholesterolemia (total cholesterol >240 mg/dl), but no patient had diabetes mellitus. The study was approved by the ethics committee of our institute, and all patients gave written informed consent.

Protocol

Nine patients were treated with benidipine 4 to 8 mg/day only and all patients achieved BP $<140/90$ mmHg within 3 months. BP and pulse rate were measured in our outpatients office every month, and PWV was monitored at 0, 3, 6, and 12 months with an automatic waveform analyzer. After completion of 1-year of treatment, benidipine was suspended for 2 weeks in all patients with informed consent. BP and PWV were reevaluated after suspension. Not only PWV, but also BP and heart rate were measured by an automatic waveform analyzer for all data analyses.

Blood samples were taken at 0, 6, and 12 months and used to measure biochemical parameters and vasoactive factors. High-sensitivity C-reactive protein (hsCRP) was measured by latex nephelometry (Dade Behring, Marburg, Germany). Plasma concentration of adrenomedullin was measured with an immunoradiometric assay kit (AM RIA Shionogi, Osaka, Japan), and endothelin-1 levels were measured using a commercially available standard radioimmunoassay kit.

Statistical Analyses

All statistical analyses were performed using StatView-J software (version 4.5; Abacus Concepts, Inc., Berkeley, USA) on a Macintosh computer. After confirmation of normal distributions for all variables, the significance of differences was evaluated by paired *t*-test or analysis of variance (ANOVA) followed by Fisher's multiple comparison tests. Relationships between variables were analyzed by simple correlation analysis and multivariate regression analysis. Data are expressed as

the mean $\pm$ SEM and a p value <0.05 was the criterion for statistical significance.

Results

Short-Term Experiment

Nicardipine administration achieved a $10.2\pm1.3\%$ decrease in SBP and an $11.9\pm1.3\%$ decrease in diastolic BP (DBP) accompanied by an $8.7\pm0.8\%$ decrease in PWV and a $13.2\pm1.5\%$ increase in heart rate. BP and PWV quickly recovered after termination of nicardipine treatment. Maximum decreases in PWV and concomitant BP changes were plotted by linear regression analysis. The changes of SBP and DBP showed a good correlation with the PWV changes, and the equations of the regression lines were $\Delta\text{PWV} = 7.258 \times \Delta\text{SBP}$ ($r=0.889$) and $\Delta\text{PWV} = 10.308 \times \Delta\text{DBP}$ ($r=0.912$), respectively. However, mean BP (MBP) changes showed the best correlation with PWV changes. The equation of the regression line was $\Delta\text{PWV} = 10.114 \times \Delta\text{MBP}$ ($r=0.913$) or $\%\Delta\text{PWV} = 0.719 \times \%\Delta\text{MBP}$ ($r=0.926$) (Fig. 1A). The changes in MBP and PWV were also well correlated in all-data analysis (Fig. 1B), but the changes in heart rate were not correlated with PWV changes ($r=0.059$, $p=0.48$). The equation of the regression line was $\Delta\text{PWV} = 7.901 \times \Delta\text{MBP}$ ($r=0.868$). MBP, but not heart rate, was a significant independent variable for PWV in multivariate regression analysis.

Long-Term Treatment

Benidipine treatment achieved stable BP control within 3 months, which was maintained until the end of the study. The average dose of benidipine at 12 months was 6.7 mg/day (4 mg for 3 patients and 8 mg for 6 patients). After 6 months, while PWV was also decreased significantly, heart rate was unchanged throughout the study (Table 1). We compared real (r-PWV) and estimated PWV (e-PWV) changes calculated from the BP decreases using the above regression formula. After 3 months, r-PWV and e-PWV showed almost the same degree of change, but the r-PWV value exceeded the e-PWV after 6 months, and the difference between r-PWV and e-PWV reached significance at 12 months (Fig. 2). The same trend was observed when BP and PWV changes were calculated as % changes ($-17.4\pm2.3\%$ for r-PWV vs. $-12.3\pm1.2\%$ for e-PWV; $p=0.028$ at 12 months). The trend became clearer when e-PWV was calculated using another formula derived from analysis of all-data (Fig. 2). Data concerning blood samples are summarized in Table 1. Interestingly, C-reactive protein (CRP) was decreased after benidipine treatment. As indicated in Table 1, other factors were not changed. Blood glucose and lipids including triglyceride, total cholesterol, high-density lipoprotein cholesterol and remnant-like lipoprotein cholesterol were also not changed (data not shown).

After suspension of benidipine for 2 weeks, BP and PWV were increased. However, these BP and PWV values

remained significantly lower than the control values (Table 1). CRP was also increased after suspension of benidipine and was no longer significantly different from the control level.

Discussion

The fully automated waveform analyzer (form PWV/ABI) can measure baPWV non-invasively and quickly, and thus has been deployed in clinical practice. The baPWV is composed of a central elastic artery component and a peripheral muscular artery component. The PWV of the peripheral arteries is greater than that of the central arteries; therefore baPWV is considerably faster than authentic carotid-femoral PWV (7). baPWV values correlated well with carotid-femoral PWV, but the two parameters did not match completely (7). Although there are some differences between the two techniques, baPWV closely correlates with BP and age of subjects and reflects risk or progression of cardiovascular disease, as does carotid-femoral PWV (4–9). The most interesting and attractive characteristic of baPWV is, however, its rapid and large response to anti-hypertensive therapy, with significant decreases in baPWV being observed within 1 month after therapy (12). Thus, the baPWV is convenient to measure, is well correlated with organ damage and is a suitable index for monitoring arterial wall stiffness. Potentially, therefore, the baPWV may represent a useful new marker of the efficacy of anti-hypertensive therapy.

We examined the acute response of baPWV during hypotension induced by intravenous administration of nicardipine, which is the sole intravenously applicable dihydropyridine CAB in Japan. Acute changes in baPWV are mainly dependent on BP, though they may be affected to a lesser degree by heart rate (19), and are independent of arterial sclerosis. Regression analysis indicated that the decrease in baPWV achieved with CAB treatment was 10 times that of MBP, or about 0.7 times the % change in MBP (Fig. 1). The impact of our data is not negligible, because our estimated influence of BP changes on baPWV was much larger than that previously reported by Ichihara *et al.* (10). In their study, the change in the ratio of baPWV/MBP in response to various anti-hypertensive drugs was between 3.9 and 5.9, vs. 10.1 in our formula. Thus, this formula could account for most of the BP influence on baPWV. However, it has been reported that angiotensin-converting enzyme inhibitors or angiotensin-II receptor antagonists caused larger baPWV reductions than CAB, for a given BP reduction (11). Therefore, the formula cannot be applied as an absolute universal standard in cases involving anti-hypertensive treatments, but it does constitute a relative guide for baPWV monitoring. Furthermore, the formula cannot be applied to correction of the influence of BP in untreated hypertensive patients, because anti-hypertensive therapy alters hemodynamic and humoral states, and may alter reactivity of the vascular wall. Indeed, while the importance of raw baPWV values is undeniable, one must be careful in interpreting baPWV changes in anti-hypertensive

treatment.

The principal finding of our study was that baPWV progressively improved during long-term successful anti-hypertensive treatment with benidipine. The improvement was maintained following correction for BP changes, after 6 months of treatment (Fig. 2). Moreover, baPWV and BP remained at lower levels 2 weeks after suspension of benidipine (Table 1). These findings strongly suggest that the arterial properties of patients are improved by benidipine in conjunction with prolonged successful BP reduction. Benetos *et al.* (20) reported that PWV increases with age even in normotensive subjects and that the increase is greatly accelerated by co-existing high BP, high heart rate and high serum creatinine. Ichihara *et al.* (10) also reported significant reductions of baPWV following intensive BP lowering in a group of hypertensive patients, but not in a group undergoing moderate BP lowering. These findings, as well as those of the present study, highlight the importance of good control of BP for preventing or reversing the hypertension-induced progression of arterial stiffness.

Benidipine and nifedipine bear a close resemblance in the clinical setting; in a multi-center trial in Japan, the two drugs demonstrated comparable BP lowering capacity and minimal influence on pulse rate (21, 22). Benidipine, however, seems to have a higher efficacy than nifedipine in coronary and glomerular arteries (23, 24). In addition to BP lowering capacity, benidipine has beneficial effects on the vascular wall: benidipine improves endothelial function *via* stimulation of nitric oxide synthesis and inhibition of endothelin-1 expression (16, 18, 25). Benidipine also has an anti-oxidative action in hypertensive patients that could contribute to the prevention of arterial sclerosis (26). In addition, the ratio of baPWV reduction to BP lowering by benidipine in the present study was larger than those in previous reports (10, 11). Thus, additional benefits of benidipine may include a contribution to the improvement of arterial properties, but further study is needed to confirm this. CRP is thought to be a predictive, causal and therapeutic marker of cardiovascular disease (27, 28), and a decrease in CRP levels has been associated with good outcomes (28). Interestingly, CAB has been shown to improve endothelial function in association with CRP decreases in the coronary circulation (29). In the present study, the decrease in CRP levels after benidipine treatment may suggest an improvement in vascular function that could have been of some benefit to the patients.

In conclusion, we have confirmed the influence of BP on baPWV in patients undergoing anti-hypertensive therapy. In that context, benidipine relieved arterial stiffness after long-term reduction in BP. Monitoring baPWV in hypertensive patients is convenient and provides useful information about the state of the arterial wall, and with further study may be applicable to estimation of cardiovascular risk.

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