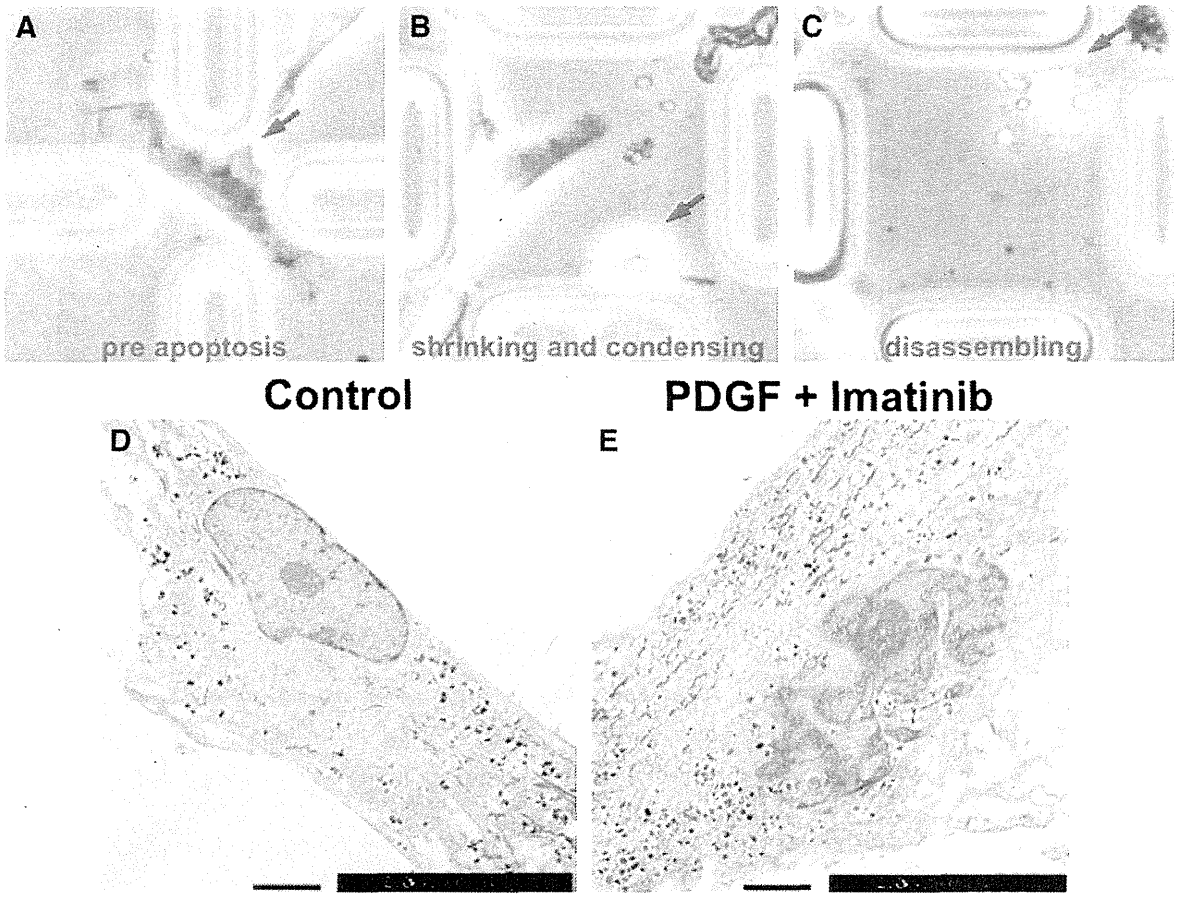

Fig. 3. Effect of imatinib on apoptosis of PASMCs in time-lapse microscopy and transmission electron microscopy. A to C, Representative images of time-lapse microscopy. IPAH-PASMCs were treated with imatinib (1 μg/mL) and PDGF-BB (10 ng/mL). Bar = 20 μm. D and E, Representative images of transmission electron microscopy. D, IPAH-PASMCs without treatment (control). E, IPAH-PASMCs treated with imatinib (1 μg/mL) and PDGF-BB (10 ng/mL). Bar = 5 μm.

way ANOVA with Fisher's PLSD test. Values of $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Inhibitory effect of imatinib on proliferation of PASMCs from IPAH patients

Treatment with imatinib inhibited PDGF-BB-induced proliferation of PASMCs from IPAH patients as assessed by ^{3}H-thymidine incorporation (n = 5–12 experiments in each cell) (Fig. 1). This result is consistent with recent findings of other investigators [10].

3.2. Effect of imatinib on apoptosis of PASMCs from IPAH patients

We performed a TUNEL assay using an ApopTag fluorescein to assess the effect of imatinib on apoptosis of PASMCs from IPAH patients. Fig. 2 shows representative cases of the TUNEL assay. TUNEL-positive cell (green) was observed after 24-hour treatment with imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) (Fig. 2D and H). However, imatinib (1 μg/mL) (Fig. 2B and F) or PDGF-BB (10 ng/mL) (Fig. 2C and G) alone did not induce apoptosis in IPAH-PASMCs. Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased TUNEL-positive cells in IPAH-PASMCs compared with the control condition in IPAH-PASMCs ($P<0.05$: 15.1 ± 5.4% versus 4.5 ± 1.3%, n = 4 or 5 experiments in each cell line) (Fig. 2J). There was no significant difference in the percentage of TUNEL-positive cells between the imatinib alone or PDGF alone condition and the control condition (Fig. 2J). There was also no significant difference between the imatinib alone, PDGF alone or both imatinib and PDGF condition and the control condition in normal PASMCs (P = NS, n = 5 experiments) (Fig. 2I).

Fig. 3A, B and C shows the apoptosis induced by the combination of imatinib (1 μg/mL) and PDGF-BB (10 ng/mL) in IPAH-PASMCs as assessed by time-lapse microscopy. A PASMC shows shrinking and condensing and finally disassembling. Fig. 3E shows a transmission electron microscopic image of an apoptotic cell in IPAH-PASMCs. Condensation of chromatin along the nuclear membrane and fragmentation of the nucleus were observed in cultured IPAH-PASMCs treated with imatinib (1 μg/mL) and PDGF-BB (10 ng/mL).

Fig. 4 shows representative cases of the caspase assay in IPAH-PASMCs. Caspase-3 and −7-active cell was observed after 24-hour treatment with imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) (Fig. 4D and H). Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased caspase-3 and −7-active cells in IPAH-PASMCs compared with the control condition ($P<0.01$: 12.4 ± 3.0% versus 2.2 ± 1.2%, n = 5 experiments in each cell line) (Fig. 4J). There was no significant difference in the percentage of caspase-3 and −7-positive cells between the imatinib alone or PDGF alone condition and the control condition in IPAH-PASMCs (Fig. 4J). There was also no significant difference between the imatinib alone, PDGF alone or both imatinib and PDGF condition and the control condition in normal PASMCs (P = NS, n = 5 experiments) (Fig. 3I).

These results show that imatinib did not induce apoptosis in normal PASMCs and quiescent IPAH-PASMCs but that imatinib had a proapoptotic effect on IPAH-PASMCs stimulated with PDGF.

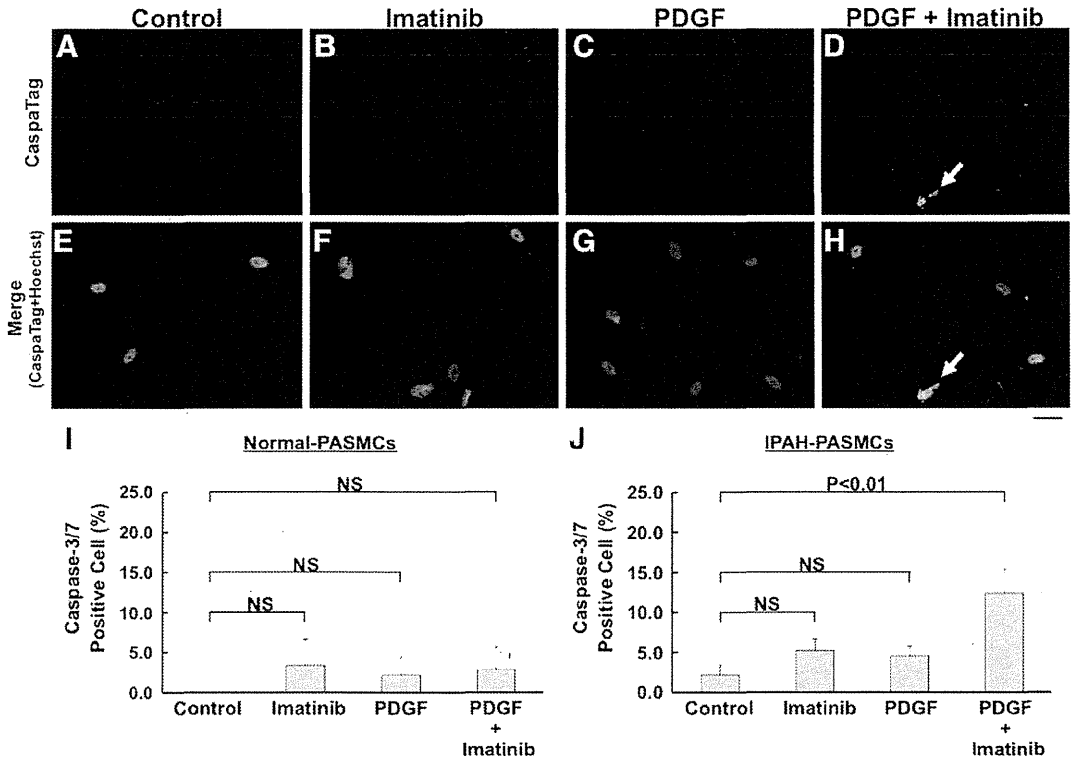

Fig. 4. Effect of imatinib on apoptosis of PASMCs in Caspase assay using a CaspaTag Caspase-3/7 in situ apoptosis detection kit. A to D, CaspaTag staining (green). E to H, Combined images (merge) of CaspaTag staining and Hoechst nuclear staining (blue). A and E, IPAH-PASMCs without treatment. B and F, IPAH-PASMCs treated with imatinib (1 μg/mL). C and G, IPAH-PASMCs treated with PDGF-BB (10 ng/mL). D and H, IPAH-PASMCs treated with imatinib and PDGF-BB. Arrow shows a caspase-3/7-positive cell (green). Bar = 500 μm. I, Effect of imatinib on apoptosis of normal PASMCs in Caspase assay. J, Effect of imatinib on apoptosis of IPAH-PASMCs in Caspase assay. Imatinib (1 μg/mL) in the presence of PDGF-BB (10 ng/mL) significantly increased caspase-positive (apoptotic) cells in IPAH-PASMCs compared with the control condition ($P<0.01$). Data are mean ± SE.

3.3. Effect of imatinib on PDGF-BB-induced phosphorylation of Akt

Western blot analysis revealed that PDGF-BB induced phosphorylation of Akt at 15 min (Fig. 5A, lanes 2 and B). Akt phosphorylation was significantly inhibited by imatinib (1 ng/mL) compared with the treatment with PDGF-BB ($P<0.05$, $n=4$ experiments) (Fig. 5A, lanes 3 and B).

Akt-I-1/2 (1 μmol/L), an Akt inhibitor, could mimic the effects of imatinib on PASMCs. Akt-I-1/2 significantly inhibited PDGF-induced proliferation of IPAH-PASMCs as assessed by ^{3}H-thymidine incorporation ($P<0.001$, $n=10$ experiments) (Fig. 5C). Akt-I-1/2 in the presence of PDGF-BB significantly increased TUNEL-positive cells ($P<0.05$, $n=5$ experiments) (Fig. 5D) and caspase-3,7-positive cells in IPAH-PASMCs ($P<0.05$, $n=5$ experiments) (Fig. 5E) compared with the control condition. These results show that the inhibition of Akt is strongly related to the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs.

4. Discussion

Two major new findings were obtained in the present study. First, imatinib did not induce apoptosis in quiescent IPAH-PASMCs and normal PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF. Second, inhibition of Akt is related to the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs.

Imatinib alone did not induce apoptosis in IPAH-PASMCs. This result is consistent with recent findings of other investigators [10]. However, the combination of imatinib and PDGF induced apoptosis. Therefore, imatinib did not induce apoptosis in quiescent IPAH-PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF. It has been reported that PDGF-A and PDGF-B mRNA levels were increased in small pulmonary arteries from patients with IPAH [10] and that serum PDGF-BB levels across the lung circulation were higher in IPAH patients [18]. Therefore, imatinib is expected to induce apoptosis in clinical settings. Further studies are needed to clarify this point.

Many signaling pathways, including ERK, p38 MAPK and Akt, are involved in proliferation and survival of PASMCs [14,19]. Akt is a member of the serine/threonine-specific kinase family known for facilitating cell survival via the inhibition of apoptotic pathways. It has been shown that PDGF stimulation transiently phosphorylates Akt and the mammalian target of rapamycin (mTOR) in PASMCs from patients with chronic thromboembolic pulmonary hypertension [19]. In our study, PDGF-BB induced phosphorylation of Akt and it was inhibited by imatinib in IPAH-PASMCs. Akt-I-1/2, an Akt inhibitor, could mimic the effects of imatinib on PASMCs. Akt is related to the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs.

Imatinib is a drug used for treating chronic myelogenous leukemia and gastrointestinal stromal tumors. However, resistance to imatinib can occur [20–22]. Not only primary resistance within the first two months but also secondary resistance develops after a median of about 2 years of treatment with the drug. Hatano et al. reported that imatinib decreases the plasma concentration of PDGF-BB in patients with PAH, while the improvement in hemodynamic parameters is transient [11]. We showed that imatinib had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF in the present study. Thus, imatinib would induce apoptosis only in the early period of treatment when plasma PDGF-BB levels are relatively high. After the PDGF levels have decreased, imatinib would not be able to induce apoptosis. Therefore, resistance to imatinib might occur in patients with pulmonary hypertension. Attention is needed in clinical use.

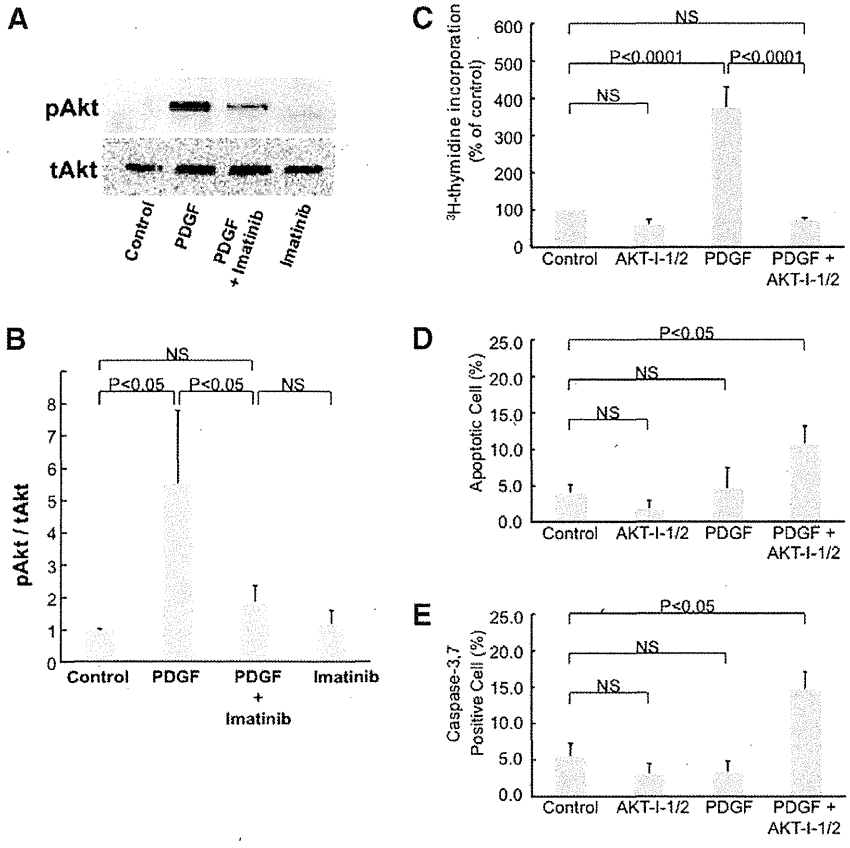

Fig. 5. Effect of imatinib on PDGF-BB-induced phosphorylation of Akt and effects of an Akt inhibitor on PDGF-BB-stimulated proliferation and apoptosis of PASMCs. A, Western blot analysis of total Akt (tAkt) and phosphorylated Akt (pAkt). PDGF-BB (10 ng/mL) induced phosphorylation of Akt at 15 min (lanes 2). Akt phosphorylation was inhibited by imatinib (1 ng/mL) (lanes 3). B, Bar graphs show semiquantitive analysis of pAkt expression level in IPAH-PASMCs. Data are mean ± SE of the intensity of the band corresponding to pAkt relative to tAkt. C, Anti-proliferative effect of Akt-I-1/2 (1 μmol/L), an Akt inhibitor, on IPAH-PASMCs stimulated with PDGF-BB (10 ng/mL). ^{3}H-thymidine incorporation was measured. Counts per minute (cpm) were expressed as a percentage of cpm of IPAH-PASMCs treated with a diluent (control). Data are mean ± SE. D, Effect of Akt-I-1/2 (1 μmol/L) on apoptosis of PASMCs in TUNEL assay by ApopTag fluorescein. E, Effect of Akt-I-1/2 (1 μmol/L) on apoptosis of PASMCs in Caspase assay using a CaspaTag Caspase-3/7 in situ apoptosis detection kit.

In conclusion, imatinib inhibited PDGF-induced proliferation of IPAH-PASMCs. Imatinib did not induce apoptosis in quiescent IPAH-PASMCs, but it had a pro-apoptotic effect on IPAH-PASMCs stimulated with PDGF. Inhibition of Akt may be important in the anti-proliferative and pro-apoptotic effects of imatinib on PDGF-stimulated IPAH-PASMCs. Modulation of PDGF signaling such as Akt is important. Inhibition of PDGF signaling by imatinib may become a useful molecular-targeted therapy for IPAH.

Conflict of interest

There are no relationships with industry.

Acknowledgments

The authors thank Kaoru Akazawa, Masayo Ohmori, and Miyuki Fujiwara for their excellent technical assistance. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [23].

References

[1] Archer S, Rich S. Primary pulmonary hypertension: a vascular biology and translational research "Work in progress". Circulation 2000;102:2781–91.
[2] Miura A, Nakamura K, Kusano KF, et al. Three-dimensional structure of pulmonary capillary vessels in patients with pulmonary hypertension. Circulation 2010;121:2151–3.
[3] Barst RJ. PDGF signaling in pulmonary arterial hypertension. J Clin Invest 2005;115:2691–4.
[4] Barst RJ, Gibbs JS, Ghofrani HA, et al. Updated evidence-based treatment algorithm in pulmonary arterial hypertension. J Am Coll Cardiol 2009;54:S78–84.
[5] Ogawa A, Nakamura K, Matsubara H, et al. Prednisolone inhibits proliferation of cultured pulmonary artery smooth muscle cells of patients with idiopathic pulmonary arterial hypertension. Circulation 2005;112:1806–12.
[6] Fujio H, Nakamura K, Matsubara H, et al. Carvedilol inhibits proliferation of cultured pulmonary artery smooth muscle cells of patients with idiopathic pulmonary arterial hypertension. J Cardiovasc Pharmacol 2006;47:250–5.
[7] Ikeda T, Nakamura K, Akagi S, et al. Inhibitory effects of simvastatin on platelet-derived growth factor signaling in pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension. J Cardiovasc Pharmacol 2010;55:39–48.
[8] Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. J Clin Invest 2005;115:2811–21.
[9] Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. N Engl J Med 2005;353:1412–3.
[10] Perros F, Montani D, Dorfmuller P, et al. Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med 2008;178:81–8.
[11] Hatano M, Yao A, Shiga T, Kinugawa K, Hirata Y, Nagai R. Imatinib mesylate has the potential to exert its efficacy by down-regulating the plasma concentration of platelet-derived growth factor in patients with pulmonary arterial hypertension. Int Heart J 2010;51:272–6.
[12] Wendel HG, De Stanchina E, Fridman JS, et al. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. Nature 2004;428:332–7.
[13] Date H, Kusano KF, Matsubara H, et al. Living-donor lobar lung transplantation for pulmonary arterial hypertension after failure of epoprostenol therapy. J Am Coll Cardiol 2007;50:523–7.
[14] Takeda M, Otsuka F, Nakamura K, et al. Characterization of the bone morphogenetic protein (BMP) system in human pulmonary arterial smooth muscle cells isolated from a sporadic case of primary pulmonary hypertension: roles of BMP type IB receptor (activin receptor-like kinase-6) in the mitotic action. Endocrinology 2004;145:4344–54.
[15] Nakamura K, Shimizu J, Kataoka N, et al. Altered nano/micro-order elasticity of pulmonary artery smooth muscle cells of patients with idiopathic pulmonary arterial hypertension. Int J Cardiol 2010;140:102–7.

[16] Kouchi H, Nakamura K, Fushimi K, et al. Manumycin A, inhibitor of ras farnesyltransferase, inhibits proliferation and migration of rat vascular smooth muscle cells. Biochem Biophys Res Commun 1999;264:915–20.
[17] Nikaido A, Tada T, Nakamura K, et al. Clinical features of and effects of angiotensin system antagonists on amiodarone-induced pulmonary toxicity. Int J Cardiol 2010;140:328–35.
[18] Selimovic N, Bergh CH, Andersson B, Sakiniene E, Carlsten H, Rundqvist B. Growth factors and interleukin-6 across the lung circulation in pulmonary hypertension. Eur Respir J 2009;34:662–8.
[19] Ogawa A, Firth AL, Yao W, et al. Inhibition of mTOR attenuates store-operated Ca^{2+} entry in cells from endarterectomized tissues of patients with chronic thromboembolic pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2009;297: L666–76.
[20] Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 2006;354:2542–51.
[21] Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347: 472–80.
[22] Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet 2004;364:1127–34.
[23] Shewan LG, Coats AJ. Ethics in the authorship and publishing of scientific articles. Int J Cardiol 2010;144:1–2.

J Pharmacol Sci **120**, 206 – 212 (2012)

Journal of Pharmacological Sciences

Full Paper

Acute Vasoreactivity Testing With Nicardipine in Patients With Pulmonary Arterial Hypertension

Yukihiro Saito[1], Kazufumi Nakamura[1,*], Katsumasa Miyaji[2], Satoshi Akagi[1], Hiroki Mizoguchi[2], Aiko Ogawa[2], Soichiro Fuke[3], Hideki Fujio[4], Takahiko Kiyooka[5], Satoshi Nagase[1], Kunihisa Kohno[1], Hiroshi Morita[1,6], Kengo F Kusano[1], Hiromi Matsubara[2], Tohru Ohe[1], and Hiroshi Ito[1]

[1]*Department of Cardiovascular Medicine,* [6]*Department of Cardiovascular Therapeutics, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama 700-8558, Japan*
[2]*Division of Cardiology, National Hospital Organization, Okayama Medical Center, Okayama 701-1192, Japan*
[3]*Department of Cardiology, Japanese Red Cross Okayama Hospital, Okayama 700-8607, Japan*
[4]*Department of Cardiology, Japanese Red Cross Society Himeji Hospital, Himeji 670-8540, Japan*
[5]*Division of Cardiology, Tokai University Oiso Hospital, Oiso 259-0198, Japan*

Received May 10, 2012; Accepted September 18, 2012

Abstract. Acute vasoreactivity testing for patients with pulmonary arterial hypertension (PAH) has been reported to be useful to identify patients with sustained beneficial response to oral calcium-channel blockers (CCBs), but there is a risk of exacerbation during the testing with oral CCBs. Therefore, we developed a testing method utilizing intravenous nicardipine, a short-acting CCB, and examined the safety and usefulness of acute vasoreactivity testing with nicardipine in PAH patients. Acute vasoreactivity testing with nicardipine was performed in 65 PAH patients. Nicardipine was administered by short-time continuous infusion (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 5 min and 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 5 min) followed by bolus injection (5 $\mu g/kg$). Hemodynamic responses were continuously measured using a right heart catheter. Acute responders were defined as patients who showed a decrease in mean pulmonary artery pressure of at least 10 mmHg to an absolute level below 40 mmHg with preserved or increased cardiac output. Two acute responders and sixty-three non-acute responders were identified. There was no hemodynamic instability requiring additional inotropic agents or death during the testing. Acute responders had good responses to long-term oral CCBs. The acute vasoreactivity testing with nicardipine might be safe and useful for identifying CCB responders in PAH patients.

Keywords: calcium-channel blocker, acute vasoreactivity testing, pulmonary arterial hypertension

Introduction

Pulmonary arterial hypertension (PAH) is a condition characterized by elevated mean pulmonary artery pressure (PAP), and the prognosis is poor in most cases (1, 2). Although we and other investigators have reported that a small proportion of patients with PAH respond to long-term calcium-channel blockers (CCBs) and have a better prognosis (3 – 6), the empiric use of CCBs in PAH is not recommended because of the risk of exacerbation (7, 8).

*Corresponding author. ichibun@cc.okayama-u.ac.jp
Published online in J-STAGE on October 30, 2012 (in advance)
doi: 10.1254/jphs.12114FP

Acute vasoreactivity testing is usually performed to predict a better prognosis and identify acute responders who are more likely to have a sustained beneficial response to oral CCBs and can be treated with these less-expensive drugs (6, 9). Although acute vasoreactivity testing is most commonly performed using inhaled nitric oxide (iNO) (10), intravenous epoprostenol (11), or intravenous adenosine (12), there are uncertainties regarding the choice of vasodilator (13).

In pulmonary arterial smooth muscle cells (PASMCs), the free Ca^{2+} concentration in the cytosol ($[Ca^{2+}]_{cyt}$) is an important determinant of contraction, migration, and proliferation. $[Ca^{2+}]_{cyt}$ in PASMCs can be increased by Ca^{2+} influx through voltage-dependent calcium channels (VDCC), receptor-operated Ca^{2+} channels (ROC), and

store-operated Ca^{2+} channels (SOC) and by Ca^{2+} release from intracellular stores via inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors. NO, epoprostenol, and adenosine suppress elevation of $[Ca^{2+}]_{cyt}$ through inhibition of ROC and SOC, which are associated with G protein–coupled receptor (GPCR), IP_3R, and transient receptor potential cation channels (TRPCs); however, CCBs suppress the elevation through inhibition of VDCCs (14).

Acute vasoreactivity testing using CCBs appears to be a reasonable method for predicting response to long-term CCBs, but the safety and efficacy of oral and intravenous CCBs for the testing have not been established in PAH patients. Occurrence of life-threatening hemodynamic compromise has often been documented in nifedipine and verapamil testing (7, 15, 16). Therefore, it is now accepted that CCBs should not be used for acute testing (17, 18).

Since oral CCBs have a long half-life, there is a risk of exacerbation due to instability of pharmacokinetics when they are used for testing (7, 16). Therefore, the development of a testing method using a short-acting intravenous CCB is needed.

Nicardipine chloride, a hypotensor available in most countries in the world, is administered to patients with hypertensive emergency, to those with acute heart failure associated with hypertension, and to those with hypertension during an operation by continuous infusion at 0.5 – 6 $\mu g \cdot kg^{-1} \cdot min^{-1}$, at 0.5 – 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and at 0.5 – 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ or bolus injection at 10 – 30 $\mu g/kg$, respectively, by reference to the Japanese package insert. The half-life of nicardipine after intravenous injection at 10 $\mu g/kg$ to a healthy adult is about 1 h, which is shorter than that of nifedipine at 10 mg per os (about 2.6 h). Therefore, we developed a testing method using short-acting intravenous nicardipine and examined the safety and usefulness of the test.

Materials and Methods

Subjects

We performed acute vasoreactivity testing using intravenous nicardipine for adult patients diagnosed as having PAH without left heart disease who had been hospitalized in our hospital from April 1999 to October 2011.

Pulmonary hypertension was defined by a resting mean PAP $\geq$ 25 mmHg during right heart catheterization (RHC) with a mean pulmonary capillary wedge pressure (PCWP) $\leq$ 15 mmHg (19).

Exclusion criteria were as follows: thromboembolic pulmonary hypertension, hemodynamic instability including cardiac index (CI) less than 2.2 $L \cdot min^{-1} \cdot m^{-2}$ and systolic blood pressure (BP) less than 80 mmHg, or having symptoms associated with low cardiac output (CO) at rest.

All of the studies were approved by the Ethics Committee of Okayama University Graduate School of Medicine, Density, and Pharmaceutical Sciences, and written informed consent was obtained from all patients before the procedure. The investigation also conformed to the principles outlined in the Declaration of Helsinki.

Baseline evaluation

Baseline evaluation included medical history, WHO functional class, physical examination, 6-min walk test, and brain natriuretic peptide (BNP). Baseline hemodynamic evaluations were performed in all patients with RHC as previously described (1, 20). Baseline hemodynamic measurements included heart rate (HR), BP, right atrial pressure (RAP), mean PAP, PCWP, and CO determined by the Fick method. CI was calculated as CO / body surface area. Total pulmonary resistance (TPR) was calculated as (mean PAP/CO) × 80.

Acute vasoreactivity testing

Acute vasoreactivity testing was performed at the time of absence of hemodynamic instability. Acute pulmonary vasodilator responsiveness was assessed by administration of nicardipine (Astellas Pharma Inc., Tokyo) with short-time continuous infusion (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 5 min and 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 5 min) followed by a bolus injection (5 $\mu g/kg$) (Fig. 1). We used continuous infusion at a low dose for a short time before the bolus injection to ensure the PH patients' safety.

Hemodynamic responses were continuously measured before, during, and after administration of nicardipine using an RHC. S_aO_2 (saturation of arterial blood) and $S_{PA}O_2$ (saturation of pulmonary arterial blood) were measured to calculate CO every 5 min. A significant acute response to nicardipine was defined as a reduction in mean PAP of at least 10 mmHg to an absolute mean PAP of less than 40 mmHg without a decrease in CO according to the American College of Chest Physicians–developed guidelines (21). Discontinuance criteria were systemic BP less than 70 mmHg, HR elevation more than 50 bpm from baseline, and/or appearance of any other constitutional symptoms.

Chronic treatment with CCB

Chronic oral CCB therapy was initiated in patients who showed significant acute pulmonary vasoreactivity as defined above.

Statistical analysis

All values are expressed as the mean ± standard deviation.

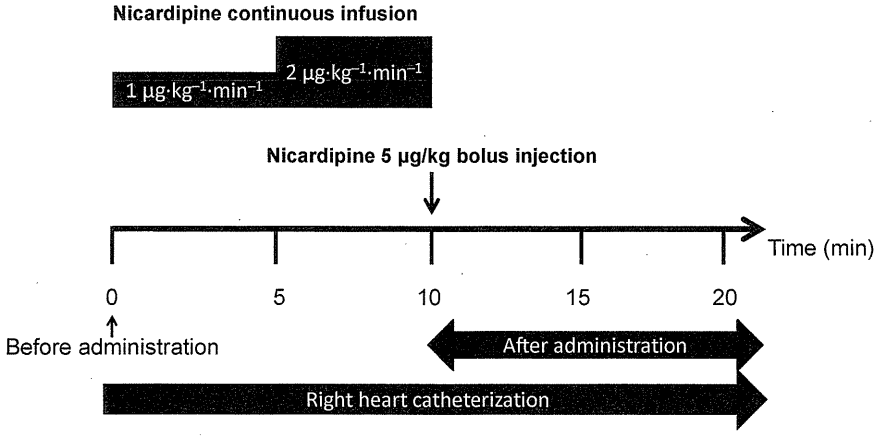

Fig. 1. Protocol of acute vasoreactivity testing with nicardipine.

Results

Study group

Sixty-five patients met the criteria of PAH and were included in the analysis. The clinical characteristics and baseline hemodynamic parameters of these 65 patients are shown in Table 1.

Responses to intravenous nicardipine and long-term CCB in acute responders

Hemodynamic changes during the testing are shown in Table 2. The values after administration shown in Table 2 were measured when maximum variation of mPAP was detected. There was no hemodynamic instability requiring additional inotropic agents or death during the testing. Two acute responders were identified.

Responder 1

A 26-year-old woman with idiopathic PAH was admitted to our hospital 4 years after diagnosis at another hospital. Beraprost sodium at a daily dose of 60 μg had been prescribed for 2 years, but shortness of breath became exacerbated and recurrent episodes of syncope during activity occurred. She was therefore referred to our hospital for treatment. Pretherapeutic hemodynamic data were as follows: HR, 65 bpm; systemic BP, 108/66/85 mmHg; PCWP, 7 mmHg; PAP, 84/35/55 mmHg; RAP, 5 mmHg; CO, 3.3 L/min; CI, 2.2 $L \cdot min^{-1} \cdot m^{-2}$; systemic vascular resistance (SVR), 1939 $dynes \cdot s \cdot cm^{-5}$; pulmonary vascular resistance (PVR), 1163 $dynes \cdot s \cdot cm^{-5}$; and TPR, 1333 $dynes \cdot s \cdot cm^{-5}$.

Unfortunately, her hemodynamic data were incomplete because the right heart catheter tip had moved down to the right ventricle from the pulmonary artery and could not be recovered. Systolic right ventricular pressure (sRVP) and saturation of right atrial blood ($S_{RA}O_2$) were therefore assessed as alternatives to systolic PAP and $S_{PA}O_2$, respectively. Her sRVP decreased to 41 mmHg from 69 mmHg and CO increased to 3.0 L/min from 2.0 L/min after administration of nicardipine, and she was therefore considered to be an acute responder.

Treatment with oral nifedipine at a daily dose of 10 mg was started, and then the daily dose was titrated up to 90 mg. Two years after starting the drug treatment, her data were as follows: HR, 64 bpm; systemic BP, 135/85/102 mmHg; PCWP, 5 mmHg; PAP, 35/10/22 mmHg; RAP, 0 mmHg; CO, 4.1 L/min; CI, 2.8 $L \cdot min^{-1} \cdot m^{-2}$; SVR, 1990 $dynes \cdot s \cdot cm^{-5}$; PVR, 331 $dynes \cdot s \cdot cm^{-5}$; and TPR, 429 $dynes \cdot s \cdot cm^{-5}$. Figure 2 shows improvement of right ventricular overload on an electrocardiogram and improvement in enlargement of cardiothoracic ratio and main pulmonary trunk on a chest X-ray. She has now achieved remission without PAH.

Responder 2

A 37-year-old woman with IPAH was admitted to our hospital with the chief complaint of shortness of breath.

Pretherapeutic hemodynamic data were as follows: HR, 65 bpm; systemic BP, 117/67/89 mmHg; PAP, 62/25/37 mmHg; RAP, 2 mmHg; CO, 6.1 L/min; CI, 3.6 $L \cdot min^{-1} \cdot m^{-2}$; SVR, 1135 $dynes \cdot s \cdot cm^{-5}$; and TPR, 482 $dynes \cdot s \cdot cm^{-5}$.

Hemodynamic changes in this acute responder were as follows: HR, 65 bpm; systemic BP, 111/60/79 mmHg; PAP, 39/15/24 mmHg; RAP, 2 mmHg; CO, 6.7 L/min; CI, 4.0 $L \cdot min^{-1} \cdot m^{-2}$; SVR, 923 $dynes \cdot s \cdot cm^{-5}$; TPR, 288 $dynes \cdot s \cdot cm^{-5}$; variation in mPAP, −13 mmHg; and variation in CO: +0.6 L/min.

Treatment with oral amlodipine at a daily dose of 2.5 mg was started. Four days after starting the drug treatment, the tricuspid regurgitation pressure gradient (TRPG) had decreased to 41 mmHg from 70 mmHg as

Table 1. Baseline characteristics

Variable	All (n = 65)
Sex: No. (%)	
Female	43 (66.2)
Male	22 (33.8)
Age: years	37 ± 17
Disease duration: month	48 ± 73
Cause of PAH: No. (%)	
Idiopathic	45 (69.2)
Congenital heart disease	9 (13.8) ASD,8; VSD,1
Connective tissue disease	5 (7.7) SLE,3; SSc,1; MCTD,1
Others	6 (9.2)
Medications: No. (%)	
CCB	8 (12.3)
Oral drug other than CCB	33 (50.8)
Epoprostenol	8 (12.3)
Clinical characteristics	
Height (cm)	161 ± 9.0
Weight (kg)	56 ± 13
Body mass index (kg/m^2)	22 ± 4.4
WHO functional class	2.4 ± 0.6
BNP (pg/mL)	130 ± 177
Hemodynamics	
Heart rate (/min)	78 ± 15
BP (s/d/m) (mmHg)	113 ± 17 / 62 ± 12 / 79 ± 13
PAP (s/d/m) (mmHg)	84 ± 26 / 36 ± 15 / 54 ± 18
Right atrial pressure (mmHg)	3.9 ± 3.6
CO (L/min) / CI (L·min^{-1}·m^{-2})	4.0 ± 1.7 / 2.5 ± 0.9
TPR (dyne·s·cm^{-5})	1223 ± 648

PAH = pulmonary arterial hypertension, CCB = calcium channel blocker, s/d/m = systolic/diastolic/mean, BNP = plasma concentration of brain natriuretic peptide, CO = cardiac output, CI = cardiac index, TPR = total pulmonary resistance, ASD = atrial septal defect, VSD = ventricular septal defect, SLE = systemic lupus erythematosus, SSc = systemic scleroderma, MCTD = mixed connective tissue disease.

Table 2. Hemodynamics during nicardipine-challenging test

	Pre	Post
Non-responder		
sBP (mmHg)	113 ± 17	106 ± 16
mPAP (mmHg)	54 ± 18	54 ± 19
CO (L/min)	4.0 ± 1.7	4.5 ± 1.8
TPR (dynes·s·cm^{-5})	1236 ± 646	1090 ± 527
Responder 1		
sBP (mmHg)	105	99
sRVP (mmHg)	69	41
CO (L/min)	2.0*	3.0*
TPR (dynes·s·cm^{-5})	-	-
Responder 2		
sBP (mmHg)	117	92
mPAP (mmHg)	37	27
CO (L/min)	6.1	7.3
TPR (dynes·s·cm^{-5})	483	297

*$S_{RA}O_2$ was substituted for $S_{PA}O_2$, and cardiac output was calculated by the Fick oxygen method. sBP = systolic blood pressure, mPAP = mean pulmonary artery pressure, sRVP = systolic right ventricular pressure, CO = cardiac output, TPR = total pulmonary resistance.

shown by ultrasonography. Now she takes amlodipine at a daily dose of 5 mg and TRPG is 53 mmHg. Figure 3 shows improvement of septal flattening during systole and TRPG by an echocardiogram.

Non-responders

Data for the 63 non-responders were as follows: baseline mPAP, 54 ± 18 mmHg; variation in mPAP, 0.9 ± 4.4 mmHg; and variation in CO, 0.4 ± 0.9 L/min.

Discussion

We performed acute vasoreactivity testing using intravenous nicardipine. The test was safe and two responders were identified in the 65 patients.

It has been demonstrated that long-term CCB responders have better prognosis than that of CCB non-responders, and CCBs are less expensive than other vasodilators (6, 22). Therefore, vasoreactivity testing to find responders is important and is required in clinical settings.

According to the ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (2009), vasodilator challenge should only be performed with short-acting, safe, and easy-to-administer drugs with no or limited systemic effects (18). Acute vasoreactivity testing is most commonly performed using inhaled iNO (10), intravenous epoprostenol (11), or intravenous adenosine (12). However, several investigators have also pointed out a problem with these agents: it is important to consider that these agents have different mechanisms of action and diverse hemodynamic effects, and their use may therefore not be interchangeable (13, 23). Since iNO vasodilates the pulmonary artery selectively and its half-life is very short (3 min), it has minimal systemic side

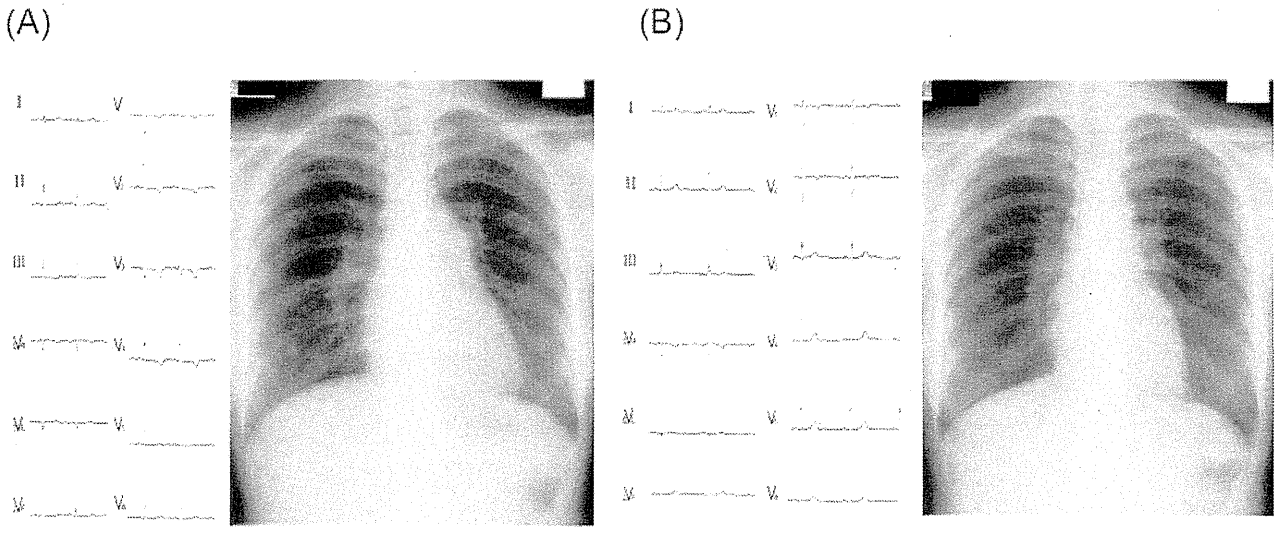

Fig. 2. Improvements in electrocardiogram and chest X-ray of responder 1. A) Before administration of nifedipine in April 1999. B) At three years after starting administration, March 2003.

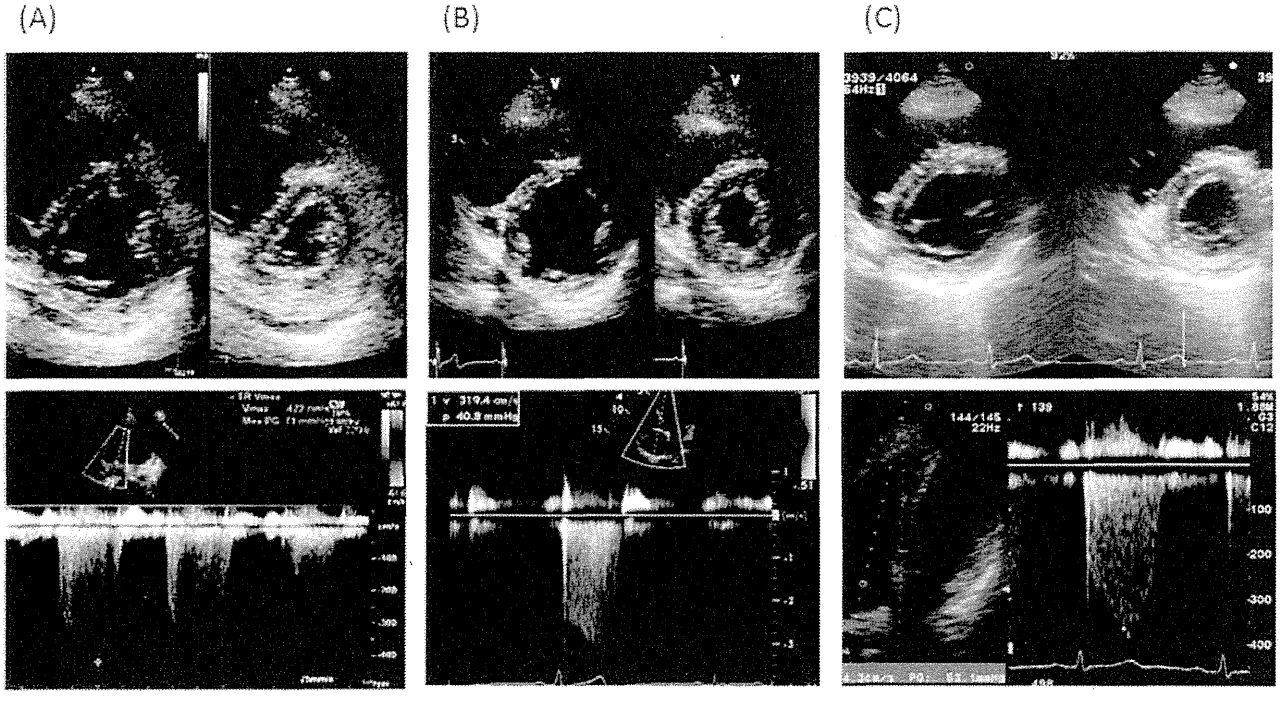

Fig. 3. Improvement in transthoracic echocardiogram of responder 2. Upper panels are parasternal short-axis view images in diastole and end-systole, and lower panels are tricuspid regurgitation (TR) velocity images. A) Before administration of amlodipine in October 2009, septal flattening at end-systole was observed (upper panel). TR velocity was 422 cm/s (lower panel) and TR pressure gradient (TRPG), which is used for estimation of systolic PAP, was 71 mmHg. B) At six days after starting administration of amlodipine at 2.5 mg in November 2009, septal flattening at end-systole was improved (upper panel). TR velocity was 319.4 cm/s (lower panel) and TRPG was 41 mmHg. C) With administration of amlodipine at 5 mg in January 2011, septal flattening at end-systole was improved (upper panel). TR velocity was 364.3 cm/s (lower panel) and TRPG was 53 mmHg.

effects (24). However, the duration of inhalation and the concentration for testing have not been standardized. Although adverse effects of iNO are very rare, to prevent leakage of NO, a delivery system, a gas cylinder, and respiratory therapy are required.

Intravenous epoprostenol revealed the patients most likely to benefit from CCB therapy; however, a favorable response to epoprostenol does not indicate that all patients will have a long-term response to CCBs (22). Epoprostenol causes frequent adverse reactions such as flushing, headache, and hypotension (24). In addition, this drug is ten-times more expensive in Japan than in America.

Adenosine is an easily available, stable, and inexpensive medication that has pulmonary vasodilatory properties. Its half-life is 5 – 10 s. When given intravenously, the short half-life allows a relatively higher plasma concentration of the agent in the pulmonary circulation rather than the systemic circulation, thus reducing systemic side effects (25). Adverse effects are palpitation, bronchospasm, hypotension, bradycardia, and atrioventricular block (26).

Acute vasoreactivity testing with the above-described drugs still fails to identify all of the patients who will have a long-term CCB response (6, 9), and it is unclear why some patients have an initial positive vasoreactivity testing but do not respond to CCBs after some time.

Furthermore, according to the ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension (2009), the use of CCBs given orally or i.v. as an acute test is discouraged due to the risk of potentially life-threatening complications; however, the statement was based on the reports in which oral nifedipine, intravenous/oral verapamil, or intravenous diltiazem was used for acute vasoreactivity testing (18, 27 – 30). We performed acute vasoreactivity testing using intravenous nicardipine. The test was safe in the 65 patients.

Nicardipine is an intravenous CCB, available not only in Japan but also in other countries, and is administrated as a hypotensor. This drug inhibits uptake of Ca^{2+} to vascular smooth muscle cells to dilate blood vessels (31). It has been reported that nicardipine has more powerful antagonism in vascular smooth muscle cells than in cardiomyocytes and is more vasoselective than other CCBs (nifedipine, verapamil, diltiazem) (32). Nicardipine, an intravenous CCB, is short-acting compared to oral CCBs. Therefore, it appeared that acute vasoreactivity testing using nicardipine could be carried out safely and might be useful for identifying long-term CCB responders more specifically than other vasodilators. Since our study was carried out in a small population, further controlled studies in larger populations are needed to confirm our results of testing including safety.

Since nicardipine was reported to induce reflex tachycardia and palpitation, we used low dosage by reference to that for patients with acute heart failure (33 – 35), and marked elevation in HR during testing was not observed in our study (Table 2). Fortunately, there were no critical hypotensive effects, and the testing could be carried out safely. However, it is often difficult to restore a patient's condition when the condition has deteriorated. Therefore, PAH patients should be closely monitored in the intensive care unit during acute vasoreactivity testing.

We did not compare this testing with other conventional vasodilator approaches and an active control group. Therefore, appropriate dosage for testing and usefulness were not adequately established. However, the mechanisms by which elevation of $[Ca^{2+}]_{cyt}$ are suppressed by nicardipine and by other drugs (NO, epoprostenol, and adenosine) are different, and addition of nicardipine testing with other vasodilator approaches might therefore raise the precision of acute vasoreactivity testing.

A positive test is observed in about 10% – 15% of patients with IPAH. Approximately half of these patients will experience long-term benefits of CCBs (23). As stated above, only a small number of patients benefit from CCBs. However, survival rate of long-term CCB responders was 97% in an average follow-up period of 7 years in a large retrospective study (n = 557) in which IPAH patients were treated with CCBs after demonstrating acute pulmonary vasoreactivity (6). Therefore, treatment with a CCB is one of the favorable and possible treatment options.

In conclusion, acute vasoreactivity testing with nicardipine might be safe for PAH patients and might be useful for identifying long-term CCB responders in PAH patients.

Acknowledgments

We thank Kaoru Akazawa, Aya Miura, Miyuki Fujiwara, and Masayo Ohmori for their excellent technical assistance.

References

1 Akagi S, Nakamura K, Miyaji K, Ogawa A, Kusano KF, Ito H, et al. Marked hemodynamic improvements by high-dose epoprostenol therapy in patients with idiopathic pulmonary arterial hypertension. Circ J. 2010;74:2200–2205.
2 Ohkita M, Tawa M, Kitada K, Matsumura Y. Pathophysiological roles of endothelin receptors in cardiovascular diseases. J Pharmacol Sci. 2012;119:302–313.
3 De Feyter PJ, Kerkkamp HJ, de Jong JP. Sustained beneficial effect of nifedipine in primary pulmonary hypertension. Am Heart J. 1983;105:333–334.
4 Saito D, Haraoka S, Yoshida H, Kusachi S, Yasuhara K, Nishihara M, et al. Primary pulmonary hypertension improved by long-term oral administration of nifedipine. Am Heart J. 1983;105:

1041–1042.
5 Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. N Engl J Med. 1992;327:76–81.
6 Sitbon O, Humbert M, Jais X, Ioos V, Hamid AM, Provencher S, et al. Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. Circulation. 2005;111: 3105–3111.
7 Partanen J, Nieminen MS, Luomanmaki K. Death in a patient with primary pulmonary hypertension after 20 mg of nifedipine. N Engl J Med. 1993;329:812–813.
8 Barst RJ, Gibbs JSR, Ghofrani HA, Hoeper MM, McLaughlin VV, Rubin LJ, et al. Updated evidence-based treatment algorithm in pulmonary arterial hypertension. J Am Coll Cardiol. 2009; 54:S78–S84.
9 Morales-Blanhir J, Santos S, de Jover L, Sala E, Pare C, Roca J, et al. Clinical value of vasodilator test with inhaled nitric oxide for predicting long-term response to oral vasodilators in pulmonary hypertension. Respir Med. 2004;98:225–234.
10 Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J. Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. Lancet. 1991; 338:1173–1174.
11 Rubin LJ, Groves BM, Reeves JT, Frosolono M, Handel F, Cato AE. Prostacyclin-induced acute pulmonary vasodilation in primary pulmonary hypertension. Circulation. 1982;66:334–338.
12 Schrader BJ, Inbar S, Kaufmann L, Vestal RE, Rich S. Comparison of the effects of adenosine and nifedipine in pulmonary hypertension. J Am Coll Cardiol. 1992;19:1060–1064.
13 Ghofrani HA, Wilkins MW, Rich S. Uncertainties in the diagnosis and treatment of pulmonary arterial hypertension. Circulation. 2008;118:1195–1201.
14 Morrell NW, Adnot S, Archer SL, Dupuis J, Jones PL, MacLean MR, et al. Cellular and molecular basis of pulmonary arterial hypertension. J Am Coll Cardiol. 2009;54:S20–S31.
15 Landmark K, Refsum AM, Simonsen S, Storstein O. Verapamil and pulmonary hypertension. Acta Med Scand. 1978;204: 299–302.
16 Sitbon O, Humbert M, Jagot JL, Taravella O, Fartoukh M, Parent F, et al. Inhaled nitric oxide as a screening agent for safely identifying responders to oral calcium-channel blockers in primary pulmonary hypertension. Eur Respir J. 1998;12:265–270.
17 Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. N Engl J Med. 2004;351:1425–1436.
18 Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009;30:2493–2537.
19 Satoh T, Saji T, Watanabe H, Ogawa S, Takehara K, Tanabe N, et al. A phase III, multicenter, collaborative, open-label clinical trial of sildenafil in Japanese patients with pulmonary arterial hypertension. Circ J. 2011;75:677–682.
20 Hashimoto K, Nakamura K, Fujio H, Miyaji K, Morita H, Kusano K, et al. Epoprostenol therapy decreases elevated circulating levels of monocyte chemoattractant protein-1 in patients with primary pulmonary hypertension. Circ J. 2004;68:227–231.
21 Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. Chest. 2007;131:1917–1928.
22 Montani D, Savale L, Natali D, Jais X, Herve P, Garcia G, et al. Long-term response to calcium-channel blockers in non-idiopathic pulmonary arterial hypertension. Eur Heart J. 2010;31: 1898–1907.
23 Tonelli AR, Alnuaimat H, Mubarak K. Pulmonary vasodilator testing and use of calcium channel blockers in pulmonary arterial hypertension. Respir Med. 2010;104:481–496.
24 Sitbon O, Brenot F, Denjean A, Bergeron A, Parent F, Azarian R, et al. Inhaled nitric oxide as a screening vasodilator agent in primary pulmonary hypertension. A dose-response study and comparison with prostacyclin. Am J Respir Crit Care Med. 1995; 151:384–389.
25 Nootens M, Schrader B, Kaufmann E, Vestal R, Long W, Rich S. Comparative acute effects of adenosine and prostacyclin in primary pulmonary hypertension. Chest. 1995;107:54–57.
26 Oliveira EC, Ribeiro AL, Amaral CF. Adenosine for vasoreactivity testing in pulmonary hypertension: a head-to-head comparison with inhaled nitric oxide. Respir Med. 2010;104:606–611.
27 Packer M, Medina N, Yushak M, Wiener I. Detrimental effects of verapamil in patients with primary pulmonary hypertension. Br Heart J. 1984;52:106–111.
28 Crevey BJ, Dantzker DR, Bower JS, Popat KD, Walker SD. Hemodynamic and gas exchange effects of intravenous diltiazem in patients with pulmonary hypertension. Am J Cardiol. 1982;49: 578–583.
29 Young TE, Lundquist LJ, Chesler E, Weir EK. Comparative effects of nifedipine, verapamil, and diltiazem on experimental pulmonary hypertension. Am J Cardiol. 1983;51:195–200.
30 Packer M, Medina N, Yushak M. Adverse hemodynamic and clinical effects of calcium channel blockade in pulmonary hypertension secondary to obliterative pulmonary vascular disease. J Am Coll Cardiol. 1984;4:890–901.
31 Terai M, Takenaka T, Maeno H. Inhibition of calcium influx in rabbit aorta by nicardipine hydrochloride (YC-93). Biochem Pharmacol. 1981;30:375–378.
32 Bristow MR, Ginsburg R, Laser JA, McAuley BJ, Minobe W. Tissue response selectivity of calcium antagonists is not due to heterogeneity of [3H]-nitrendipine binding sites. Br J Pharmacol. 1984;82:309–320.
33 Kumada T, Kawai C, Sasayama S, Kinoshita M, Kawamura K, Kusukawa R, et al. [Clinical efficacy of nicardipine by intravenaous infusion in patients with acute heart gailure. A multicenter randomized double-blind placebo-controlled trial.] Jpn Pharmacol Ther. 1995;23:375–398. (text in Japanese with English abstract)
34 Kinoshita M, Kawai C, Kumada T, Kawamura K, Kusukawa R. [Acute effects of a single dose of intravenous administration of nicardipine hydrochloride in patients with acute heart failure.] Early phase II study. Jpn Pharmacol Ther. 1995;23:345–356. (text in Japanese with English abstract)
35 Hirota Y, Kawai C, Hori R, Okumura K, Kinoshita M, Kumada T, et al. [Intravenous nicardipine infusion for the treatment of acute heart failure. A double blind study for the optimal dose determination (late phase II study).] Jpn Pharmacol Ther. 1995; 23:357–373. (text in Japanese with English abstract)

Heart Vessels (2013) 28:179–187
DOI 10.1007/s00380-011-0224-3

ORIGINAL ARTICLE

Evaluation of exercise capacity using wave intensity in chronic heart failure with normal ejection fraction

Yoichi Takaya · Manabu Taniguchi · Motoaki Sugawara · Saori Nobusada · Kengo Kusano · Teiji Akagi · Hiroshi Ito

Received: 27 July 2011 / Accepted: 8 December 2011 / Published online: 4 February 2012

Abstract Impaired exercise capacity has been found in patients with diastolic dysfunction with preserved systolic function. Although conventional transthoracic echocardiography (TTE) provides useful clinical information about systolic and diastolic cardiac function, its capability to evaluate exercise capacity has been controversial. The inertia force of late systolic aortic flow is known to have a tight relationship with left ventricular (LV) performance during the period from near end-systole to isovolumic relaxation. The inertia force and the time constant of LV pressure decay during isovolumic relaxation can be estimated noninvasively using the second peak (W_2) of wave intensity (WI), which is measured with an echo-Doppler system. We sought to determine whether W_2 is associated with exercise capacity in patients with chronic heart failure with normal ejection fraction (HFNEF) and to compare its ability to predict exercise capacity with parameters obtained by conventional TTE including tissue Doppler imaging. Sixteen consecutive patients with chronic HFNEF were enrolled in this study. Wave intensity was obtained with a color Doppler system for measurement of blood velocity combined with an echo-tracking system for detecting changes in vessel diameter. Concerning conventional TTE, we measured LV ejection fraction (EF), peak velocities of early (*E*) and late (*A*) mitral inflow using pulse-wave Doppler, and early (Ea) and late (Aa) diastolic velocities using tissue Doppler imaging. Left ventricular EF, *E*/*A* ratio, Ea, and *E*/Ea ratio did not correlate with exercise capacity, whereas W_2 significantly correlated with peak VO_2 ($r = 0.54$, $p = 0.03$), VE/VCO_2 slope ($r = -0.53$, $p = 0.03$), and $\Delta VO_2/\Delta WR$ ($r = 0.56$, $p = 0.02$). W_2 was associated with exercise capacity in patients with chronic HFNEF. In conclusion, W_2 is considered to be clinically more useful than conventional TTE indices for evaluating exercise capacity in patients with chronic HFNEF.

Keywords Wave intensity · Second peak · Exercise capacity · Echocardiography · Heart failure

Y. Takaya (✉) · K. Kusano · H. Ito
Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: takayayoichi@yahoo.co.jp

M. Taniguchi · T. Akagi
Division of Cardiac Intensive Care Unit, Okayama University Hospital, Okayama, Japan

M. Sugawara
Department of Medical Engineering, Himeji Dokkyo University, Himeji, Japan

S. Nobusada
Division of Central Clinical Laboratory, Okayama University Hospital, Okayama, Japan

Introduction

Left ventricular (LV) diastolic abnormalities with preserved systolic function contribute to symptoms of heart failure [1, 2]. Cardiopulmonary exercise testing provides an objective means of assessing exercise capacity, and has become an important clinical tool with which to predict outcome in chronic heart failure patients [3–5]. Most investigators have focused on cardiac factors that predict exercise capacity, and it has been suggested that LV diastolic function is useful for determining exercise capacity, because an excessive rise in pulmonary capillary wedge pressure is the main cardiac cause of exertional dyspnea [6, 7].

In the previous study, Sugawara et al. [8] proposed the concept that the inertia force of aortic flow near end-ejection, which is generated by the ability of the left ventricle to actively stop blood flow, causes a rapid decrease in LV pressure, i.e., swift end-systolic unloading, producing a much smaller LV end-systolic volume. The resulting greater elastic recoil force brings faster LV relaxation. Thus, the inertia force may be linked with exercise capacity because it has a tight relationship with LV performance during the period from near end-systole to isovolumic relaxation. In the clinical setting, the inertia force can be estimated by the concept of wave intensity (WI), which is obtained noninvasively using an echo-Doppler system. Wave intensity is a hemodynamic index, which can evaluate the working condition of the heart interacting with the arterial system [9–11], and carotid arterial WI has two positive peaks. The first peak (W_1) of WI occurs in the early phase of LV ejection, and the second peak (W_2) occurs near end-ejection. During the period of W_2, the left ventricle actively stops aortic blood flow by generating forward traveling expansion (suction) waves. Therefore, we considered that W_2 obtained noninvasively may be useful for predicting exercise capacity.

Recent studies have emphasized that tissue Doppler imaging, which provided an estimate of LV diastolic function, i.e., the ratio of early transmitral velocity to tissue Doppler mitral annular early diastolic velocity (*E*/Ea), correlated with exercise capacity [12, 13]. However, several studies reported the limitations of current Doppler echocardiography for estimating LV diastolic function [14–16].

Therefore, we hypothesized that W_2 may be more strongly linked with exercise capacity compared with conventional transthoracic echocardiography (TTE) including tissue Doppler imaging. In the present study, we sought to determine whether W_2 is associated with exercise capacity in patients with chronic heart failure with normal ejection fraction (HFNEF), and to assess its ability to predict exercise capacity in comparison with parameters obtained by conventional TTE including tissue Doppler imaging.

Patients and methods

Study population

The study population consisted of 16 consecutive patients with chronic HFNEF (LV ejection fraction ≧45%) [17]. There were 10 males and 6 females with a mean age of 59 ± 13 years (range 39–73 years). The causes of heart failure were ischemia in 4 patients (25%) and nonischemia in 12 patients (75%). Current medication included β-blockers (44%), angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (56%), digitalis (31%), and diuretics (31%). Patients were excluded if they had uncontrolled heart failure, angina, severe native valvular disease, or chronic lung disease. Patients were also excluded if the symptom-limited exercise test was not possible because of other non-cardiac conditions. All patients gave written informed consent.

Definition of time-normalized wave intensity

Wave intensity was originally defined as the product of ΔP and ΔU, where ΔP and ΔU are the changes in blood pressure P and velocity U, during constant short time intervals [9, 10]. This original wave intensity depends on the sampling interval, Δt, which makes it difficult to compare data taken at different sampling rates. Therefore, we normalized WI as the product of the derivatives of P and U with respect to time, dP/dt and dU/dt [11, 18]. Thus, the time-normalized WI is introduced:

$$\mathrm{WI} = (dP/dt) \cdot (dU/dt),$$

where dP/dt and dU/dt are the derivatives of P and U with respect to time. This time-normalized WI has the same property as the original wave intensity: if WI >0, the changes in pressure and velocity caused by the forward wave are greater than those caused by the backward wave, and vice versa [9, 10].

Measurements of wave intensity

Blood pressure waveforms are needed to calculate WI. Previous studies have demonstrated that arterial pressure waveforms and diameter-change waveforms are similar [19–21]. The similarity between carotid arterial pressure waveforms measured with a catheter-tipped micromanometer and carotid arterial diameter-change waveforms measured by echo tracking in humans has been reported [21] (Fig. 1). Therefore, using systolic and diastolic pressure measured with a cuff-type manometer applied to the upper arm, we calibrated the maximum and minimum values of a diameter-change waveform and used it as a surrogate for a blood pressure waveform. We used the system consisting of a color Doppler system (Prosound α10; Aloka, Tokyo, Japan) with a 7.5-MHz linear array probe, an echo-tracking subsystem, which is installed in the color Doppler system, and a personal computer [22, 23]. The echo-tracking subsystem automatically measures arterial diameter change with a precision of one-sixteenth of the ultrasound wavelength. The blood flow velocity averaged along the Doppler beam crossing the artery is measured by using range-gated color Doppler signals. This system uses different ultrasound beams for diameter change and for blood flow velocity measurements that can be manipulated independently. The two beams intersect so

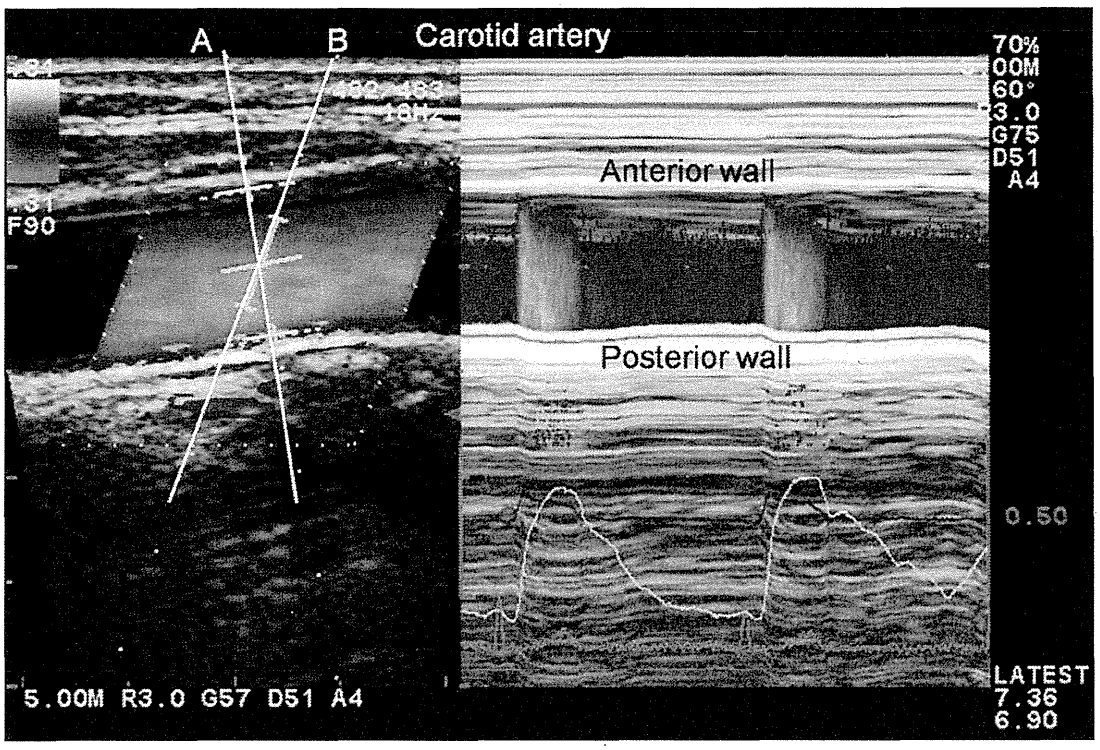

Fig. 1 Simultaneous measurements of diameter-change waveform and blood flow velocity. View on the monitor during the measurements **Left** color Doppler/B-mode long-axis view of the common carotid artery. *Line A* and *B* indicate the ultrasonic beam for echo-tracking and for blood flow velocity measurement, respectively. By setting the tracking positions displayed as small orange bars on the echo tracking beam (*line A*) to arterial walls, echo tracking automatically starts. The blood flow velocity averaged along the Doppler beam (*line B*) crossing the artery was measured using range-gated color Doppler signals (color of the blood flow velocity is removed to indicate the tracking bars clearly). **Right** the diameter-change waveform, which is calculated by subtracting the distance to the near wall from that to the far wall, and the velocity waveform are displayed on the M-mode view

that the center of the two tracking lines on the diameter-measuring beam and the center of the range gate of the velocity-measuring beam are superimposed. The diameter-change waveform, which is calculated by subtracting the distance to the anterior wall from that to the posterior wall, and the blood flow velocity waveform are displayed on the M-mode view. The sphygmomanometer-measured blood pressure data are entered for calibration. Five consecutive beats are ensemble-averaged to obtain a representative waveform. The maximum and minimum values of the diameter-change waveform are calibrated by systolic and diastolic blood pressure, and WI indices, W_1 and W_2, are calculated automatically. A stiffness parameter β is also calculated from the measured data.

In this study, the measurements of WI were made with the patients in the supine position after 15 min rest. Data were acquired from the right common carotid artery at about 2 cm proximal to the bifurcation to avoid any influence of the complex flow in the carotid sinus. In the long-axis scanning, optimal images were best achieved by positioning and orienting the probe so that clear and parallel delineation of the intima-media complex at both the anterior and posterior walls could be seen. The echo-tracking beam was steered so that it was orthogonal (90°) to the arterial walls. Echo tracking was performed just outside of the intima-media complex where stable echo tracking was possible. The color Doppler beam was steered so that the angle between the beam and flow direction was less than 60°. Recording of diameter, velocity, and WI were made.

The reproducibility of the present WI measurement system has been reported, but the variability of W_2 is higher than those of other WI indices [23]. Therefore, WI was measured more than six times just before cardiopulmonary exercise testing, and the mean value in each patient was calculated. The analysis was performed by two independent experienced investigators who were blinded to the data of cardiopulmonary exercise testing.

Measurements of conventional transthoracic echocardiography

All patients underwent TTE just before cardiopulmonary exercise testing. All measurements were averaged from

three consecutive cycles. Left ventricular end-diastolic and end-systolic volumes were calculated by the modified Simpson rule, and the standard formula was applied to give LV ejection fraction (EF). Mitral inflow was analyzed for peak early diastolic (*E*) and late diastolic (*A*) velocities, *E*/*A* ratio, and deceleration time of *E* velocity by pulse-wave Doppler. The early (Ea) and late (Aa) diastolic velocities were measured by tissue Doppler imaging, obtained from the apical four-chamber view. Ea septal mitral annulus velocities were measured, and the dimensionless mitral *E*/Ea ratio was calculated.

Cardiopulmonary exercise testing

All patients underwent symptom-limited exercise tests on an upright bicycle ergometer using a ramp protocol (15 W/min) with simultaneous respirator gas analysis. Patients were encouraged to exercise to exhaustion or to a respiratory exchange ratio $\geqq$1.09. Blood pressure and heart rate were measured every minute. A 12-lead electrocardiogram was continuously monitored during exercise testing. Breathed gas was continuously collected by a gas analyzer to analyze oxygen uptake (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE). Exercise duration was defined as the time from the start of exercise until its cessation from dyspnea or leg fatigue. Peak VO_2 was defined as the highest VO_2 value achieved at peak exercise after reaching the respiratory compensation point. The gradient of the VE–VCO_2 relationship (VE/VCO_2 slope) and the oxygen uptake to work rate ($\Delta VO_2/\Delta WR$) were also determined.

Statistical analysis

Continuous data are presented as mean ± standard deviation (SD). Discrete data are presented as numbers or frequencies of occurrence. Pearson's correlation coefficients were used to evaluate the correlation between WI or conventional TTE and exercise capacity. A probability value (*p*) of less than 0.05 was considered statistically significant.

Results

Clinical characteristics of the 16 patients are summarized in Table 1. The measurements of conventional TTE, WI, and cardiopulmonary exercise testing are summarized in Table 2. The mean value of LVEF was 59 ± 10%. The mean value of *E*/Ea ratio was 9.9 ± 2.5, and 13 (81%) of the patients had a value of *E*/Ea ranging from 8 to 15. The mean values of W_1 and W_2 were 7170 ± 4180 mmHg m/s^3 and 1550 ± 590 mmHg m/s^3. Regarding cardiopulmonary exercise testing, the mean exercise time was 9.0 ± 2.1 min. The peak VO_2 averaged 19.0 ± 4.3 ml/min/kg, which corresponded to 62 ± 14% of the maximal age- and sex-predicted peak VO_2. Dyspnea, rather than leg fatigue, was the most frequent cause of exercise cessation. No patients had angina, significant ischemic ST abnormalities in the electrocardiogram, or severe arrhythmia during cardiopulmonary exercise testing.

The correlations of conventional TTE and WI with exercise capacity are summarized in Table 3. Left ventricular EF did not correlate with the parameters of exercise capacity (exercise time, peak load, peak VO_2, VE/VCO_2 slope, or $\Delta VO_2/\Delta WR$) (Fig. 2a–c). There were also no correlations between *E*/*A* ratio, Ea (Fig. 2d–f) or *E*/Ea ratio (Fig. 2g–i) and the parameters of exercise capacity. Regarding WI, there were no correlations between W_1 and the parameters of exercise capacity. However, W_2 was significantly correlated with exercise time ($r = 0.63$, $p = 0.009$), peak load ($r = 0.58$, $p = 0.02$), peak VO_2 ($r = 0.54$, $p = 0.03$), VE/VCO_2 slope ($r = -0.53$, $p = 0.03$), and $\Delta VO_2/\Delta WR$ ($r = 0.56$, $p = 0.02$) (Fig. 2j–l). W_2 was associated with exercise capacity in patients with chronic HFNEF.

Discussion

In the present study, LVEF, *E*/*A* ratio, Ea and *E*/Ea ratio did not correlate with the parameters of exercise capacity, while W_2, which is connected to the inertia force, significantly correlated with peak VO_2, VE/VCO_2 slope, and $\Delta VO_2/\Delta WR$. We found that W_2 more strongly correlated

Table 1 Patient characteristics

Variables	
Age (years)	59 ± 13
Male	10 (63)
Body mass index (kg/m^2)	24.8 ± 5.9
Clinical diagnosis	
Ischemia	4 (25)
Nonischemia	12 (75)
Systolic blood pressure (mmHg)	125 ± 17
Diastolic blood pressure (mmHg)	77 ± 13
Heart rate (beats/min)	68 ± 10
Medical treatment	
β-Blockers	7 (71)
ACE inhibitors or ARBs	9 (56)
Digitalis	5 (31)
Diuretics	5 (31)

Data are presented as mean ± SD or numbers (percentage)

ACE angiotensin-converting enzyme, *ARB* angiotensin receptor blocker

Table 2 Measurements of transthoracic echocardiography, wave intensity, and cardiopulmonary exercise testing

Variables	
Transthoracic echocardiography	
LVEF (%)	59 ± 10
LVDd (mm)	51 ± 7
LVDs (mm)	35 ± 8
E (m/s)	0.64 ± 0.19
A (m/s)	0.68 ± 0.14
E/A ratio	1.0 ± 0.3
Deceleration time (ms)	246 ± 59
Ea (cm/s)	6.6 ± 1.7
E/Ea ratio	9.9 ± 2.5
Wave intensity	
W_1 (mmHg m/s^3)	7170 ± 4180
W_2 (mmHg m/s^3)	1550 ± 590
Stiffness parameter β	9.3 ± 3.8
Cardiopulmonary exercise testing	
Exercise time (min)	9.0 ± 2.1
Peak load (W)	101 ± 42
Peak VO_2 (ml/min/kg)	19.0 ± 4.3
VE/VCO_2 slope	26.4 ± 5.4
$\Delta VO_2/\Delta$WR	7.5 ± 1.4

Data are presented as mean ± SD

LVEF left ventricular ejection fraction, *LVDd* left ventricular diastolic dimension, *LVDs* left ventricular systolic dimension, *E* peak early transmitral diastolic velocity, *A* peak late transmitral diastolic velocity, *Ea* peak early diastolic velocity of medial mitral annulus, W_1 first peak of wave intensity, W_2 second peak of wave intensity, VO_2 oxygen uptake, *VE/*VCO_2 *slope* gradient of the ventilation to carbon dioxide production, $\Delta VO_2/\Delta WR$ oxygen uptake to work rate

with exercise capacity than with conventional TTE. This suggests the potential of W_2 in the clinical setting for predicting exercise capacity in patients with chronic HFNEF.

It has been established that patients with heart failure have impaired exercise tolerance, which is the predictor of mortality [24]. Although the mechanisms of exercise intolerance are multifactorial, they depend at least in part on the pump function of the heart, i.e., the response of cardiac output to exercise. The increase in cardiac output during exercise is the primary determinant of exercise tolerance in patients with heart failure [25, 26]. Several studies have shown that more than 50% of elderly patients who present with symptoms of heart failure have preserved LV systolic function [27–29]. Recently, LV diastolic dysfunction has been considered to be a major factor in limiting exercise capacity by raising diastolic pressures and compromising LV filling [6, 7]. In the clinical setting, Doppler echocardiography can characterize LV diastolic function through a combination of measurements, which show evidence of slowed LV relaxation, increased LV stiffness, or abnormality of LV filling pressure. In particular, *E*/Ea ratio measured by tissue Doppler echocardiography has been shown to be useful in estimating LV filling pressure, one component of diastolic function that reflects pulmonary capillary wedge pressure [30]. However, it remains controversial whether parameters of resting conventional TTE, including tissue Doppler echocardiography, can predict exercise capacity [12, 13, 31]. There are several limitations in estimating LV diastolic function with tissue Doppler echocardiography [14–16]. The patients with a value of *E*/Ea >15 are considered to have diagnostic evidence for elevated LV filling pressure [15], whereas the patients with a wide range of *E*/Ea values ($8 < E/\text{Ea} < 15$) are required to obtain an LV filling pressure estimate from additional investigations, including left atrial size, mitral filling time, deceleration time of *E* velocity, pulmonary venous flow velocity, and pulmonary artery systolic pressure. No single parameter of Doppler echocardiography has yielded a robust criterion for LV diastolic function. Furthermore, there are technical limitations including angle dependency, signal noise, signal drifting, spatial resolution, sample volume, and tethering artifacts. In the present study, patients with *E*/Ea values ranging from 8 to 15 were included. This may have brought about our result that *E*/Ea ratio did not associate with exercise capacity.

Wave intensity is a hemodynamic index, which can evaluate the working condition of the heart interacting with the arterial system [9–11]. Several studies have indicated clinical usefulness of WI [23, 32–36]. Wave intensity is obtained in the carotid artery using an echo-Doppler system, and it has two positive peaks. W_1, which occurs in the early phase of LV ejection, correlates with the maximum rate of LV pressure rise (max. d*P*/d*t*) and reflects LV contractile function [32], whereas W_2 occurs near end-ejection. During the period of W_2, both aortic pressure and velocity are decreasing; therefore, the forward-traveling waves during this period are expansion (suction) waves. In the previous study, Sugawara et al. [8] proposed the concept that the inertia force of blood flowing out of the left ventricle toward the aorta, which must be decelerated to a standstill, causes a rapid decrease in LV pressure and generates suction waves in the left ventricle near end-ejection. The inertia force causes swift end-systolic unloading of the left ventricle, producing a much smaller LV end-systolic volume and greater elastic recoil force. Thus, the inertia force contributes to the deceleration of aortic flow and produces a greater W_2. In the clinical setting, Ohte et al. [32] reported that W_2 was significantly higher in patients with the inertia force than in those without the inertia force. From these findings, W_2 is connected to the inertia force and should have a close

Table 3 Correlations of transthoracic echocardiography and wave intensity with exercise capacity

	Exercise time	Peak load	Peak VO_2	VE/VCO_2 slope	$\Delta VO_2/\Delta WR$
Systolic blood pressure	0.06	−0.05	−0.05	−0.20	−0.02
Diastolic blood pressure	0.25	0.13	−0.28	−0.49	−0.20
Heart rate	−0.02	0.06	−0.28	0.05	0.09
Transthoracic echocardiography					
LVEF	−0.07	−0.09	−0.06	0.07	−0.02
LVDd	0.26	0.25	0.18	−0.14	0.25
LVDs	0.27	0.28	0.16	−0.16	0.12
E	0.01	0.04	0.14	0.14	−0.01
A	−0.35	−0.48	-0.45	0.36	−0.33
E/*A* ratio	0.12	0.28	0.29	−0.20	−0.13
Deceleration time	−0.15	−0.28	-0.44	−0.15	−0.30
Ea	0.25	0.34	0.41	−0.06	0.17
E/Ea ratio	−0.26	−0.38	−0.23	0.20	−0.21
Wave intensity					
W_1	0.21	0.20	0.15	−0.38	0.24
W_2	0.63**	0.58*	0.54*	−0.53*	0.56*
Stiffness parameter β	−0.27	−0.25	−0.35	0.08	0.05

r value is shown. $*p < 0.05$, $**p < 0.01$

LVEF left ventricular ejection fraction, *LVDd* left ventricular diastolic dimension, *LVDs* left ventricular systolic dimension, *E* peak early transmitral diastolic velocity, *A* peak late transmitral diastolic velocity, *Ea* peak early diastolic velocity of medial mitral annulus, W_1 first peak of wave intensity, W_2 second peak of wave intensity, VO_2 oxygen uptake, *VE/*VCO_2 *slope* gradient of the ventilation to carbon dioxide production, $\Delta VO_2/\Delta WR$ oxygen uptake to work rate

relationship with LV performance during the period from near end-systole to isovolumic relaxation.

Several mechanisms by which W_2 can influence exercise capacity in patients with chronic HFNEF have been proposed. LV diastolic function is a main factor limiting exercise capacity by an excessive rise in pulmonary capillary wedge pressure [6, 7]. In late systole, LV pressure rapidly declines until left atrial pressure exceeds that of the left ventricle, leading to the onset of early filling [37]. W_2 itself is closely correlated with the indices of LV relaxation calculated invasively, such as the maximum rate of LV pressure decay and the time constant of LV pressure decay during isovolumic relaxation [32]. Moreover, early diastolic relaxation is quantified by the rate of pressure decay and is also influenced by late systolic arterial loading [38, 39]. The inertia force causes rapid end-systolic unloading of the left ventricle, tending to reduce LV end-systolic volume and increase elastic recoil force [8]. Furthermore, LV contraction and ejection dynamics are intimately linked to LV relaxation from the viewpoint of cardiac mechanics [40–42]. The inertia force is a crucial parameter whereby LV systolic function delivers its effect on left ventricle early diastolic performance. The greater inertia force is produced from better LV systolic function [8, 43]. Thus, the concept of hemodynamically induced inertia force may contribute to the further understanding of LV contraction–relaxation coupling. Indeed, the previous study indicated that a higher inertia force in a resting condition was associated with greater exercise capacity [8]. The inertia force was enhanced during exercise in patients who had a relatively large inertia force in a resting condition [8] because sympathetic stimulation and tachycardia produced a downward shift of the early diastolic portion of the LV pressure–volume loop [44]. A good heart enhances its function by generating and then utilizing the inertia force more effectively during exercise. These findings support our results that W_2, which corresponds to the inertia force, correlated with exercise capacity in patients with chronic HFNEF.

Our study had several limitations. First, only 16 patients from a single center were involved. Study of large patient populations from various centers is needed to confirm our data. Second, we enrolled patients who received medical treatments. In particular, the effects of vasodilators on the vascular system are known. Niki et al. [45] evaluated the effects of nitroglycerin on the cardiovascular system using WI, and indicated that W_1 and stiffness parameter β increased after nitroglycerin administration, but W_2 did not change significantly. However, it is possible that these medical treatments cause different results of the association between W_2 and exercise capacity. Finally, exercise capacity is also influenced by noncardiac factors, such as

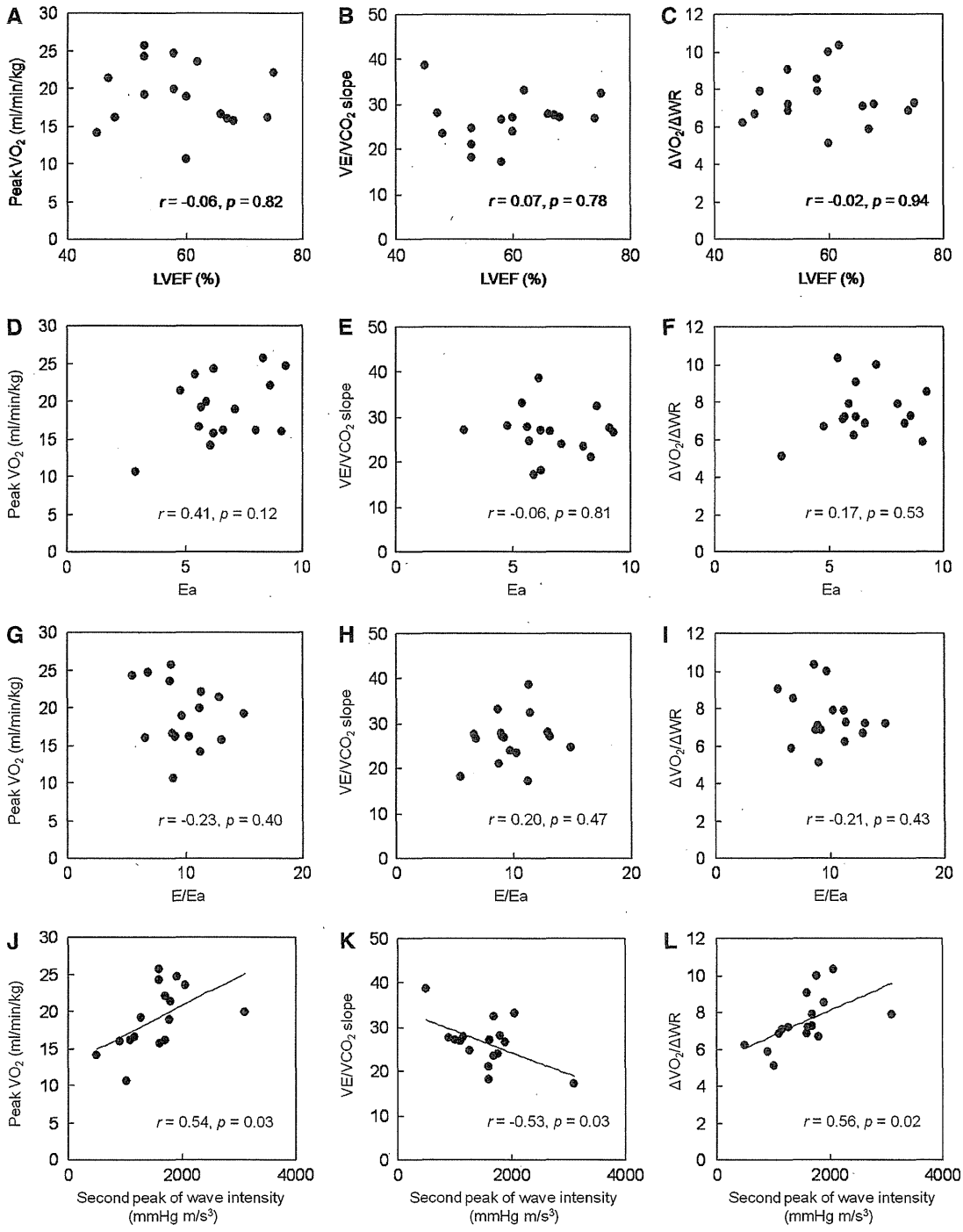

Fig. 2 Correlations of LVEF (**a–c**), Ea (**d–f**), *E*/Ea ratio (**g–i**), and the second peak of wave intensity (**j–l**) with exercise capacity. Significant correlations between the second peak of wave intensity and peak VO_2 (**j**), VE/VCO_2 slope (**k**), and $\Delta VO_2/\Delta WR$ (**l**) are observed. *LVEF* left ventricular ejection fraction, *E* peak early transmitral diastolic velocity. *Ea* peak early diastolic velocity of medial mitral annulus, VO_2 oxygen uptake, *VE/VCO*$_2$ *slope* gradient of the ventilation to carbon dioxide production; *ΔVO*$_2$*/ΔWR* oxygen uptake to work rate

age, gender, pulmonary function, hemoglobin content, and skeletal muscle. These factors might have influenced exercise capacity in this study.

In conclusion, W_2 can be noninvasively obtained using an echo Doppler and echo-tracking system in the clinical setting, and may be more useful to predict exercise

capacity in comparison with conventional TTE in patients with chronic HFNEF.

References

1. Kitzman DW, Little WC, Brubaker PH, Anderson RT, Hundley WG, Marburger CT, Brosnihan B, Morgan TM, Stewart KP (2002) Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. JAMA 288:2144–2150
2. Redfield MM, Jacobsen SJ, Burnett JC, Mahoney DW, Bailey KR, Rodeheffer RJ (2003) Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. JAMA 289:194–202
3. Beniaminovitz A, Mancini DM (1999) The role of exercise-based prognosticating algorithms in the selection of patients for heart transplantation. Curr Opin Cardiol 4:114–120
4. Franciosa JA (1984) Exercise testing in chronic congestive heart failure. Am J Cardiol 53:1447–1450
5. Lipkin DP (1987) The role of exercise testing in chronic heart failure. Br Heart J 58:559–566
6. Packer M (1990) Abnormalities of diastolic function as a potential cause of exercise intolerance in chronic heart failure. Circulation 81(suppl III):78–86
7. Little WC, Kitzman DW, Cheng CP (2000) Diastolic dysfunction as a cause of exercise intolerance. Heart Fail Rev 5:301–306
8. Sugawara M, Uchida K, Kondoh Y, Magosaki N, Niki K, Jones CJH, Sugimachi M, Sunagawa K (1997) Aortic blood momentum—the more the better for the ejecting heart in vivo? Cardiovasc Res 33:433–446
9. Parker KH, Jones CJH (1990) Forward and backward running waves in the arteries: analysis using the method of characteristics. ASME J Biomech Eng 112:322–326
10. Parker KH, Jones CJH, Dawson JR, Gibson DG (1998) What stops the flow of blood from the heart? Heart Vessels 4:241–245
11. Ramsey MW, Sugawara M (1997) Arterial wave intensity and ventriculoarterial interaction. Heart Vessels 12:128–134
12. Okura H, Inoue H, Tomon M, Nishiyama S, Yoshikawa T, Yoshida K, Yoshikawa J (2000) Impact of Doppler-derived left ventricular diastolic performance on exercise capacity in normal individuals. Am Heart J 139:716–722
13. Skaluba SJ, Litwin SE (2004) Mechanisms of exercise intolerance: insights from tissue Doppler imaging. Circulation 109:972–977
14. Mullens W, Borowski AG, Curtin RJ, Thomas JD, Tang WH (2009) Tissue Doppler imaging in the estimation of intracardiac filling pressure in decompensated patients with advanced systolic heart failure. Circulation 119:62–70
15. Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, Tajik AJ (2000) Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: a comparative simultaneous Doppler-catheterization study. Circulation 102:1788–1794
16. Carsten T, Walter JP (2009) Doppler echocardiography yields dubious estimates of left ventricular diastolic pressures. Circulation 120:810–820
17. Cohn JN, Johnson G (1990) Heart failure with normal ejection fraction. The V-HeFT Study. Veterans Administration Cooperative Study Group. Circulation 81(2 Suppl):III 48–III 53
18. Jones CJH, Sugawara M, Kondoh Y, Uchida K, Parker KH (2002) Compression and expansion wavefront travel in canine ascending aortic flow: wave intensity analysis. Heart Vessels 16:91–98
19. Barnett GO, Mallos AJ, Shapiro A (1961) Relationship of aortic pressure and diameter in the dog. J Appl Pysiol 16:545–548
20. Patal DJ, de Freitas FM, Greenfield JC Jr, Fry DL (1963) Relationship of radius to pressure along the aorta in living dogs. J Appl Physiol 18:1111–1117
21. Sugawara M, Niki K, Furuhata H, Ohnishi S, Suzuki S (2000) Relationship between the pressure and diameter of the carotid artery in humans. Heart Vessels 15:49–51
22. Harada A, Okada A, Sugawara M, Niki K (2000) Development of a non-invasive real time measurement system of wave intensity. Proc IEEE Ultrasonics Symp 2:1517–1520
23. Niki K, Sugawara M, Chang D, Harada A, Okada T, Sakai R, Uchida K, Tanaka R, Mumford CE (2002) A new noninvasive measurement system for wave intensity: evaluation of carotid arterial wave intensity and reproducibility. Heart Vessels 17: 12–21
24. Yellin EL, Nikolic S, Frater RW (1990) Left ventricular filling dynamics and diastolic function. Prog Cardiovascular Dis 32:247–271
25. Florea VG, Mareyev VY, Achilov AA, Popovici MI, Caots AJ, Belenkov YN (1999) Central and peripheral components of chronic heart failure: determinants of exercise tolerance. Int J Cardiol 70:51–56
26. Franciosa JA, Leddy CL, Wilen M, Schwartz DE (1984) Relation between hemodynamic and ventilatory response in determining exercise capacity in severe congestive heart failure. Am J Cardiol 53:127–134
27. Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, Levy D (1999) Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction. J Am Coll Cardiol 33:1948–1955
28. Cowie MR, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, Suresh V, Sutton GC (1999) Incidence and aetiology of heart failure: a population-based study. Eur Heart J 20:421–428
29. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, Gong Y, Liu PP (2006) Outcome heart failure with preserved ejection fraction in a population-based study. N Engl J Med 355:260–269
30. Nagueh SF, Middleton KJ, Kopelen HA, Zoghbi WA, Quinones MA (1997) Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressure. J Am Coll Cardiol 30:1527–1533
31. Witte KK, Nikitin NP, De Silva R, Cleland JG, Clark AL (2004) Exercise capacity and cardiac function assessed by tissue Doppler imaging in chronic heart failure. Heart 90:1144–1150
32. Ohte N, Narita H, Sugawara M, Niki K, Okada T, Harada A, Hayano J, Kimura G (2003) Clinical usefulness of carotid arterial wave intensity in assessing left ventricular systolic and early diastolic performance. Heart Vessels 18:107–111
33. Sugawara M, Niki K, Ohte N, Okada T, Harada A (2009) Clinical usefulness of wave intensity analysis. Med Biol Eng Comput 47:197–206
34. Larsson M, Bjällmark A, Lind B, Balzano R, Peolsson M, Winter R, Brodin LA (2009) Wave intensity wall analysis: a novel noninvasive method to measure wave intensity. Heart Vessels 24:357–365
35. Bjällmark A, Larsson M, Nowak J, Lind B, Hayashi SY, do Nascimento MM, Riella MC, Seeberger A, Brodin LÅ (2011) Effects of hemodialysis on the cardiovascular system: quantitative analysis using wave intensity wall analysis and tissue velocity imaging. Heart Vessels 26:289–297
36. Liu J, Cao TS, Duan YY, Yang YL, Yuan LJ (2011) Effects of cold pressor-induced sympathetic stimulation on the mechanical properties of common carotid and femoral arteries in healthy males. Heart Vessels 26:214–221
37. Borlaug BA, Kass DA (2006) Mechanisms of diastolic dysfunction in heart failure. Trends Cardiovasc Med 16:273–279
38. Kawaguchi M, Hay I, Fetics B, Kass DA (2003) Combined ventricular systolic and arterial stiffening in patients with heart

failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations. Circulation 107:714–720
39. Yano M, Kohno M, Kobayashi S, Obayashi M, Seki K, Ohkusa T, Miura T, Fujii T, Matsuzaki M (2001) Influence of timing and magnitude of arterial wave reflection on left ventricular relaxation. Am J Physiol Heart Circ Physiol 280:H1846–H1852
40. Brutsaert DL, Sys SU (1989) Relaxation and diastole of the heart. Physiol Rev 69:1228–1315
41. Burkhoff D, de Tombe PP, Hunter WC (1993) Impact of ejection on magnitude and time course of ventricular pressure-generating capacity. Am J Physiol 265:H899–H909
42. Brutsaert DL, Sys SU (1997) Diastolic dysfunction in heart failure. J Card Fail 3:225–242
43. Yoshida T, Ohte N, Narita H, Sakata S, Wakami K, Asada K, Miyabe H, Saeki T, Kimura G (2006) Lack of inertia force of late systolic aortic flow is a cause of left ventricular isolated diastolic dysfunction in patients with coronary artery disease. J Am Coll Cardiol 48:983–991
44. Cheng CP, Igarashi Y, Little WC (1992) Mechanism of augmented rate of left ventricular filling during exercise. Circ Res 70:9–19
45. Niki K, Sugawara M, Chang D, Harada A, Okada T, Tanaka R (2005) Effects of sublingual nitroglycerin on working conditions of the heart and arterial system: analysis using wave intensity. J Med Ultrason 32:145–152