Original Article

Effects of heart rate reduction with ivabradine on vascular stiffness and endothelial function in chronic stable coronary artery disease

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Introduction: Epidemiological and clinical studies have shown a relevant association between heart rate and cardiovascular mortality. Experimental studies identified vascular effects of heart rate reduction with the *If* channel inhibitor ivabradine. Therefore, the effects of heart rate reduction on endothelial function and indices of arterial stiffness were examined in patients with stable coronary artery disease in a prospective, placebo-controlled clinical crossover study.

Methods and results: Twenty-three patients (18 men and 5 women) with a resting heart rate (HR) of at least 70 beats per minute (bpm) and stable coronary artery disease were enrolled in this study. In a cross-over design, all patients were treated with ivabradine (Iva, 7.5 mg b.i.d.) and placebo for 6 months each. Iva reduced heart rate by 11.4 bpm (Iva 58.8 ± 8.2 bpm vs. placebo 70.2 ± 8.3 bpm, $P < 0.0001$). Augmentation index (AIx75), carotid–femoral pulse wave velocity (cfPWV) and central aortic blood pressure were measured using applanation tonometry (SphygmoCor). HRR by Iva increased AIx75 by 12.4% (Iva $24.3 \pm 10.5\%$ vs. placebo $21.3 \pm 10.1\%$, $P < 0.05$) and reduced cfPWV by 14.1% (Iva 6.3 ± 1.7 m/s vs. placebo 7.3 ± 1.4 m/s, $P < 0.01$). Iva increased mean central blood pressure by 7.8% (Iva 107.5 ± 15.4 mmHg vs. placebo 99.1 ± 12.2 mmHg, $P < 0.001$). Endothelial function was determined measuring the flow-mediated vasodilation (FMD) of the brachial artery. HRR by Iva increased FMD by 18.5% (Iva $7.3 \pm 2.2\%$ vs. placebo $6.0 \pm 2.0\%$, $P < 0.001$). Aortic distensibility was characterized by MRI. HRR by Iva increased aortic distensibility by 33.3% (Iva 0.003 ± 0.001/mmHg vs. placebo 0.002 ± 0.010/mmHg, $P < 0.01$) and circumferential cyclic strain by 37.1% (Iva 0.062 ± 0.027 vs. placebo 0.039 ± 0.018, $P < 0.0001$).

Conclusion: Heart rate reduction with Iva increased endothelium-dependent vasodilation and reduced arterial stiffness in patients with stable CAD. These findings corroborate and expand the results collected in experimental studies and indicate the importance of heart rate as a determinant of vascular function.

Keywords: arterial stiffness, central aortic pressure, coronary artery disease, endothelial function, ivabradine, resting heart rate

Abbreviations: ACE, angiotensin converting enzyme; AIx75, augmentation index; ARB, angiotensin receptor blocker; AV, atrioventricular; b.i.d., bis in die (twice daily); BP, blood pressure; bpm, beats per minute; CAD, coronary artery disease; CD, diastolic circumference; cfPWV, carotid–femoral pulse wave velocity; CS, systolic circumference; CVD, coronary vessel disease; FMD, flow-mediated dilation; NMD, nitromediated dilation; GTN, glyceroletrinitrate; HR, heart rate; HRR, heart rate reduction; ICD, implantable cardioverter-defibrillator; Iva, ivabradine; NO, nitric oxide; PP, pulse pressure; PWA, pulse wave analysis; PWV, pulse wave velocity; RAAS, renin–angiotensin–aldosterone system; RCT, randomized controlled trial; SEM, standard error of the mean

INTRODUCTION

Heart rate (HR) is a key determinant of cardiovascular function and is closely connected with the pathogenesis of cardiovascular disease [1]. Elevated resting HR affects vascular integrity and contributes to endothelial dysfunction and atherosclerosis in experimental studies [2–4]. Epidemiological studies and clinical trials revealed associations between resting HR and cardiovascular outcomes and increased resting HR predicts cardiovascular events in the general population [5,6] and in individuals with cardiovascular disease [7–9]. Consequently, HR qualified as a therapeutic target and, therefore,

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selective HR reduction was the primary focus of several randomized controlled trials (RCTs) in heart failure and coronary artery disease (CAD) populations. However, whereas HR reduction with the If channel inhibitor ivabradine (Iva) reduced outcomes in heart failure [10,11], a targeted HR reduction did not affect coronary events and outcome in patients with established stable CAD without heart failure [12,13]. Paradoxically, in hypertensive individuals, HR reduction achieved primarily via beta blockade was found to increase risk [14].

Numerous effects of pharmacological HR reduction have been documented in animal models and clinical studies. In dyslipidemic mice, selective HR reduction by Iva improved endothelial function [3]. Moreover, HR reduction affects central arterial properties and hemodynamics. In transgenic mice, HR reduction with Iva improved ventricular–arterial coupling, increased central aortic distensibility and reduced pulse wave velocities [15,16]. However, in addition to those potentially protective effects, conflicting results have been detected in clinical investigations with regard to aortic hemodynamics and central aortic pressure, a prognostic variable in hypertension. Heart rate reduction with atenolol or with Iva increased central aortic blood pressure in hypertension and in stable CAD [17,18].

Several studies demonstrated a direct relationship between resting HR and arterial stiffness, an independent predictor of cardiovascular morbidity and mortality [19]. Cross-sectional and longitudinal data from population-based cohorts showed a positive relationship between HR and central arterial stiffness [20–22]. Experimental studies investigating acute effects of cardiac pacing on indices of arterial stiffness detected heterogenous results. Although acute changes in HR did not affect aortic stiffness in individuals without underlying CVD [23], this was the case in individuals with implanted pacemakers showing that HR increased by pacing resulted in blood pressure–independent increases in pulse wave velocity [24]. To date, effects of pharmacologic HR reduction on arterial stiffness have not been studied in humans. Therefore, we investigated whether selective HR reduction with the *If* inhibitor Iva affects measures of arterial stiffness and endothelial function in patients with chronic stable coronary artery disease in a randomized, placebo-controlled, double-blind, crossover, single-center clinical study.

METHODS

Patient population

This randomized, placebo-controlled, double-blind, crossover study was conducted at a single tertiary center at the University Hospital of the Saarland (University of the Saarland; ClinicalTrials.gov Identifier: NCT 01768585, Eudra CT Number: 2012-001989-15). Participants were selected after an inpatient stay involving coronary angiography because of chronic stable angina. Patients were randomly allocated to placebo or Iva (7.5 mg b.i.d.; Servier, Neuilly-sur-Seine, France) in a crossover design. The main inclusion criteria were angiographically proven coronary heart disease, sinus rhythm and a resting HR of at least 70 bpm. Exclusion criteria included acute coronary syndromes, need for coronary bypass grafting, history of heart failure, stroke or diabetes, pacemaker or ICD treatment, previous treatment with Iva and presence of contraindication for the study drug. All included patients gave written informed consent for participation and data collection. The study was approved by the local ethics committee (ID Nr 240/11) and was performed according to the declaration of Helsinki.

Study protocol

The study design is depicted in Fig. 1. Patients were screened during an index hospitalization for coronary

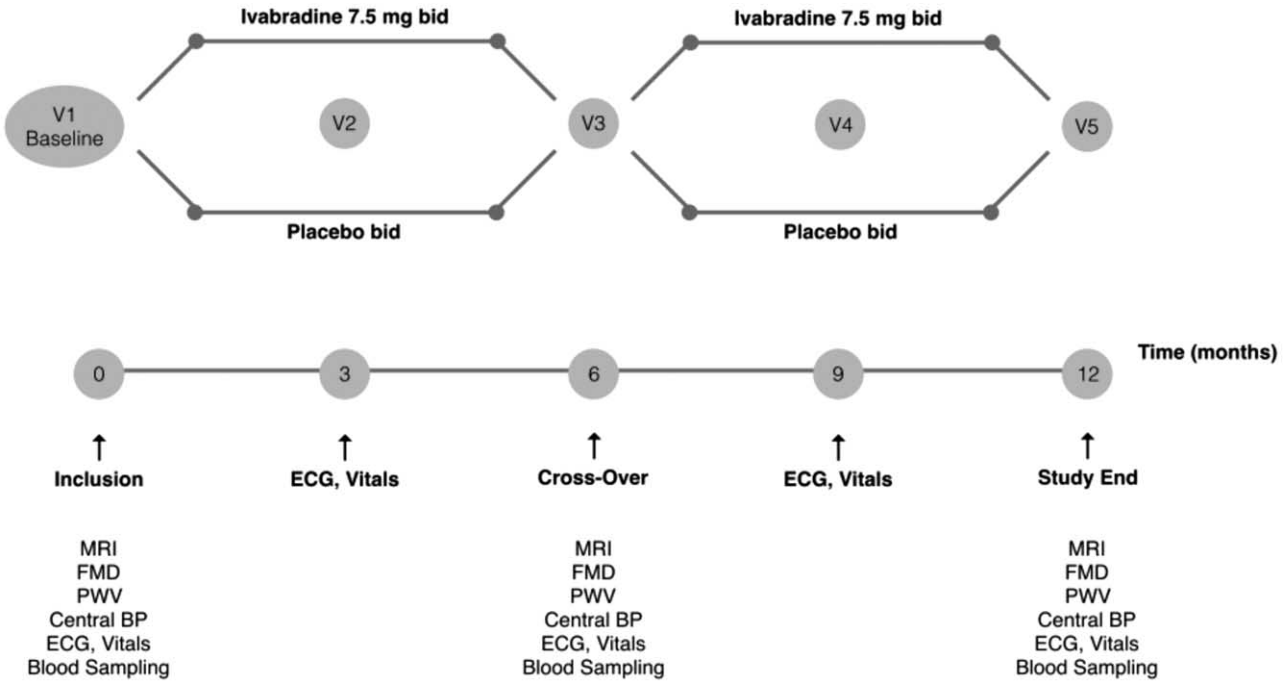

FIGURE 1 Study design. bid, bis in die (twice daily); BP, blood pressure; FMD, flow-mediated dilation; PWV, pulse wave velocity.

angiography and study inclusion was performed after discharge. The baseline visit included determination of vital signs, a 12-lead ECG, collection of venous blood samples, MRI of the thoracic aorta, applanation tonometry and determination of flow-mediated dilatation (FMD) of the brachial artery. Patients were randomized to placebo or Iva (7.5 mg b.i.d.) in a double-blind fashion and received treatment for 6 months.

Investigators and patients were blinded to the study drug and treatment sequence allocation. Blinding and allocation to placebo or Iva was ensured by the hospital pharmacist. Concomitant baseline medications including beta blockers were continued throughout the study. Follow-up visits at months 3 and 9 included a 12-lead ECG and measurement of vital signs. After the first 6 months of treatment, all measurements included in the baseline visit were repeated and participants crossed over to placebo or Iva depending on the first treatment allocation.

Endpoints and evaluations

Resting HR was recorded from a resting 12-lead ECG performed during the baseline visit and during every follow-up visit after 10 min of rest in supine position. Brachial blood pressure measurement was performed using a certified device (Omron 705-IT; Kyoto, Japan) at every single visit. Additional measurements were obtained before pulse wave analysis and MRI. Measurements were performed after a 10-min rest in the supine position at the left upper arm. A mean value was calculated from three separate measurements.

Applanation tonometry was performed using a SphygmoCor device (SphygmoCor CPV; AtCor, Medical West Ryde, Australia) to determine both augmentation index (AIx75), augmentation pressure and pulse wave velocity (PWV). The applanation probe was positioned on the particular artery (radial, femoral, carotid) and the central aortic waveform was calculated by the device software using the generalized transfer function (Fourier analysis) [25]. Radial waveforms were calibrated using brachial SBP and DBP measured before applanation. AIx75 and augmentation pressure were derived using pulse wave analysis of the radial artery. Pulse wave velocity (cfPWV) was measured at the carotid and femoral artery. The distance (d) between the two recording sites was registered and the PWV was calculated as: PWV = d (m)/t (s).

Endothelial function was characterized as FMD and nitroglycerin-induced dilation (NMD) of the right brachial artery and was examined by two-dimensional high-resolution ultrasonic imaging according to the guidelines of the American College of Cardiology [26]. All examinations were conducted by the same blinded investigator (A.L.H.) on the same ultrasound machine (GE Vivid-I; General Electric Healthcare, Niskayuna, New York, USA) throughout the study. All measurements were performed in the morning after an overnight fasting period in a noise protected room of constant temperature. Patients rested in a supine position for at least 10 min before the examination and remained supine until the final recording was acquired. Using a linear array transducer (12 MHz LRS; General Electric Healthcare) and a stereotactic clamp, longitudinal sections of the right brachial artery were obtained above the cubital fossa. The brachial diameter (B) was measured during the baseline visit using an automatic edge-detection software (Brachial Analyzer, Medical Imaging Applications, Iowa City, Iowa, USA). Simultaneous pulse-wave Doppler velocity signals were acquired with an Insonation angle of less than 60°. Reactive hyperemia was induced by 5 min of forearm occlusion using a blood pressure cuff at pressures of 250 mmHg. After cuff release pulse-wave Doppler velocity signals were recorded for a period of 5 s. Sixty seconds after cuff deflation, longitudinal images of the brachial artery were obtained (H). FMD was calculated as the percent change in brachial artery diameter in response to hyperemia (FMD (%) = $(H-B)/B \times 100$). After 10 min of rest, another baseline diameter was obtained followed by sublingual application of 0.4 mg glycerol trinitrate (GTN) and additional scans after 4 min in order to determine NMD (N). NMD was expressed as the percentage of change relative to the baseline diameter (NMD (%) = $(N-B)/B \times 100$).

MRI studies were performed on a 1.5 Tesla scanner (Magnetom Aera; Siemens Healthcare, Erlangen, Germany) using a phased-array body surface coil and ECG synchronization. For this purpose, retrospectively ECG-gated cine SSFP sequences were acquired perpendicular to the ascending aorta at the level of the right pulmonary artery (TR/TE = 58.1/1.3 ms, FA = 60, FOV = 20 × 20 cm, matrix = 192 × 192).

Quantitative evaluation of the data included assessment of the cross-sectional vessel area at end-systole and end-diastole at the level of the right pulmonary artery. For quantitative evaluation, the acquired image data was transferred to an external workstation and analyzed in a blinded manner using image evaluation software (OsiriX, Apple Inc., Cupertino, California, USA). Blood pressure was measured in triplicate during the MRI acquisition using a fully automatic device (Maglife Serenity, Schiller AG, Baar, Switzerland). Aortic distensibility was calculated using the formula: distensibility = (end-systolic aortic area − end-diastolic aortic area)/(end-diastolic aortic area × pulse pressure) [27]. Aortic circumferential cyclic strain was calculated using the formula: circumferential cyclic strain = $0.5 \times \{[(CS^2/CD^2) - 1]\}$ where CS is the systolic circumference and CD is the diastolic circumference of the ascending aorta [28]. With regard to previous findings in apolipoprotein-E-knock-out mice, we regarded aortic distensibility as the main endpoint.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 7.0a (GraphPad Software Inc, San Diego, California, USA), two-sided P values below 0.05 were considered statistically significant. Data are expressed as mean values ± standard deviation (SD) for continuous variables and percentages for categorical variables. Treatment effects were assessed by paired t-tests comparing quantitative values and ANOVA for multiple comparisons. Post hoc comparisons were performed with the Newman–Keuls test.

RESULTS

Study population

Nineteen male and 7 female patients who met the inclusion criteria were enrolled in this study. Data of 23 patients (18 men and 5 women) who completed the trial protocol were

TABLE 1. Baseline characteristics of the study population

Number of patients	26
Sex (male/female)	19/7
Age (years)	61.77 ± 9.40
Age range (years)	51–82
Current smoker (%)	17 (65.38%)
BMI (kg/m^2)	29.29 ± 4.73
Concomitant diseases	
Arterial hypertension (%)	25 (96,15%)
Hyperlipoproteinemia (%)	24 (92.31%)
Asymptomatic peripheral vascular disease (%)	1 (3.85%)
Cardiovascular parameters	
Heart rate (bpm)	80.15 ± 7.52
SBP (mmHg)	137.87 ± 18.42
DBP (mmHg)	80.13 ± 11.47
Concomitant medication	
β-blocker (%)	19 (73.07%)
ACE/AT II receptor inhibitor (%)	25 (96.15%)
Calcium channel inhibitor (%)	4 (15.38%)
Acetylsalicylic acid (%)	25 (96.15%)
Statin (%)	24 (92.31%)
Nitrate (%)	3 (11.54%)

Values are mean ± SD or *n* (%). ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; AT, angiotensin.

integrated into the final analysis. Three patients were excluded from the study after randomization [one patient because of newly diagnosed malignancy (lung carcinoma), two patients who withdrew consent]. Two patients suffered from serious tremor prohibiting applanation tonometry. Therefore, the tonometry data report 21 study participants.

Baseline characteristics of the study population ($n = 26$) are shown in Table 1. The mean age was 61.8 ± 9.4 years and the mean BMI was 29.3 ± 4.7 kg/m^2. Participants exhibited a distinct cardiovascular risk profile. Ninety-six percent had arterial hypertension, 92% had dyslipoproteinemia and 65% were current smokers. At baseline and during the study, 92% of the study participants were treated with a statin, 96% with RAAS blockers and 96% with platelet inhibitors. Seventeen patients (74%) received a beta-blocker. Six patients (26%) exhibited contraindications [e.g. bronchial asthma, asymptomatic peripheral arterial occlusive disease (PAOD), COPD GOLD II/III] Concomitant medication was not changed during the study.

Heart rate

In the treatment arm receiving placebo first, mean HR was 76.4 ± 8.0 bpm at baseline and 78.0 ± 8.9 bpm in the treatment arm receiving Iva first (Table 2). The mean on-treatment HR with Iva was 58.8 ± 8.2 bpm corresponding to a heart rate reduction (HRR) of 15.7 bpm (21.1%, $P < 0.0001$). Mean on-treatment in the placebo group was 70.2 ± 8.3 bpm ($P < 0.05$) (Table 4). SBPs or DBPs were not affected by Iva. In patients receiving concomitant beta-blockade, HR was not affected significantly compared with patients not taking beta-blockade (Table 3). The individual on-treatment HRs and blood pressures are shown in Table 2 and Table 4.

TABLE 3. Baseline and on-treatment heart rate with regard to concomitant beta-blocker co-medication

Heart rate	On beta-blocker	No beta-blocker	*P* value
Baseline	76.26 ± 8.07	79.50 ± 8.91	0.25
During Iva	57.47 ± 6.27	62.50 ± 6.80	0.11
During placebo	69.53 ± 7.89	72.08 ± 4.18	0.46

Iva, ivabradine.

Brachial flow-mediated dilation

Effects of Iva on brachial FMD are reported in Fig. 2a and Table 4. The diameter of the brachial artery at rest (before hyperemic stimuli) was consistent throughout the study (baseline 4.39 ± 0.74 mm, Iva 4.39 ± 0.74 mm, placebo 4.38 ± 0.74 mm, $P = 0.9974$). After cuff release, diameters increased to 5.96 ± 2.11 mm at baseline, 7.31 ± 2.15 mm during Iva and 5.95 ± 2.00 mm during placebo ($P = 0.0481$). The mean baseline FMD of the entire study population was 6.0 ± 2.1%. Baseline FMD values did not differ statistically between the two treatment groups (Iva 5.9 ± 1.7 vs. placebo 5.9 ± 2.3, $P = 0.9273$). Heart rate reduction with Iva increased FMD to 7.3 ± 2.2 ($P < 0.001$ vs. placebo). Endothelium-independent vasodilation after sublingual application of GTN was similar between Iva-treated and placebo-treated individuals.

Central blood pressure, pulse wave velocity and augmentation index

The mean baseline central blood pressure of both treatment groups was 98.3 ± 12.2 mmHg and did not differ between groups (Iva first 99.6 ± 8.5 vs. placebo first 97.4 ± 14.7, $P = 0.6795$;Fig. 2b and Table 4). Treatment with Iva significantly increased mean central blood pressure compared with placebo (Iva 107.5 ± 15.4 vs. placebo 99.1 ± 12.2, $P < 0.001$). Pulse wave velocity was similar in both treatment arms at baseline (Iva first 7.2 ± 1.6 vs. placebo first 7.7 ± 1.4, $P = 0.4789$). Heart rate reduction reduced carotid–femoral PWV by 14.1% (Iva 6.3 ± 1.7 vs. placebo

TABLE 2. Baseline and on-treatment heart rate and blood pressure

	Ivabradine first ($n = 10$)			Placebo first ($n = 13$)		
	HR	BP		HR	BP	
		Systolic	Diastolic		Systolic	Diastolic
Baseline	78.0 ± 8.9	132.1 ± 17.8	81.9 ± 11.7	76.4 ± 8.0	142.3 ± 18.3	78.8 ± 11.6
On-treatment phase 1	58.4 ± 10.4 ****	135.1 ± 20.8	78.6 ± 11.2	68.5 ± 7.7 ***	146.7 ± 19.3	81.6 ± 12.7
On-treatment phase 2	72.4 ± 8.7 *	134.8 ± 13.3	83.1 ± 7.5	59.1 ± 6.2 ****	151.5 ± 20.7	80.0 ± 12.1

BP, blood pressure; HR, heart rate. *P* value refers to comparison vs. baseline.
*P less than 0.05.
**P less than 0.01.
***P less than 0.001.
****P less than 0.0001.

TABLE 4. Effects of heart rate reduction with ivabradine at baseline and follow-up

Parameter	Ivabradine ($n=23$)	Placebo ($n=23$)
Heart rate (bpm)		
Baseline	74.48 ± 8.69	
End of treatment	58.78 ± 8.21	70.20 ± 8.26
Difference from baseline to end	−15.7 ± 8.45 ****	−4.28 ± 8.48 *
Brachial SBP (mmHg)		
Baseline	137.87 ± 18.42	
End of treatment	144.37 ± 22.10	141.50 ± 17.78
Difference from baseline to end	6.5 ± 20.26	3.8 ± 18.10
Brachial DBP (mmHg)		
Baseline	80.13 ± 11.47	
End of treatment	79.39 ± 11.61	82.24 ± 10.65
Difference from baseline to end	−0.74 ± 11.54	2.11 ± 11.06
FMD (%)		
Baseline	5.97 ± 2.11	
End of treatment	7.30 ± 2.15	5.95 ± 2.00
Difference from baseline to end	1.33 ± 2.13 ***	−0.05 ± 2.01
NMD (%)		
Baseline	10.57 ± 3.64	
End of treatment	11.39 ± 3.77	10.94 ± 3.77
Difference from baseline to end	0.82 ± 3.71	0.37 ± 3.71
Central SBP (mmHg)		
Baseline	124.33 ± 15.05	
End of treatment	138.24 ± 21.04	125.71 ± 15.60
Difference from baseline to end	13.91 ± 18.05 ****	1.38 ± 15.33
Central DBP (mmHg)		
Baseline	81.38 ± 10.51	
End of treatment	86.29 ± 11.95	81.29 ± 10.54
Difference from baseline to end	4.91 ± 11.23**	−0.09 ± 10.53
cfPWV (m/s)		
Baseline	7.45 ± 1.43	
End of treatment	6.30 ± 1.71	7.33 ± 1.42
Difference from baseline to end	−1.15 ± 1.57*	−0.12 ± 1.43
AIx75 (%)		
Baseline	22.29 ± 10.02	
End of treatment	24.29 ± 10.51	21.29 ± 10.10
Difference from baseline to end	2.00 ± 10.23*	−1.00 ± 10.06
Aortic distensibility (10^{-3}/mmHg)		
Baseline	1.773 ± 1.169	
End of treatment	2.619 ± 1.249	1.743 ± 1.019
Difference from baseline to end	0.846 ± 1.209**	−0.03 ± 1.319
Circumferential cyclic strain		
Baseline	0.038 ± 0.022	
End of treatment	0.062 ± 0.027	0.039 ± 0.018
Difference from baseline to end	0.024 ± 0.025****	0.001 ± 0.02

AIx75, augmentation index; cfPWV, carotid–femoral pulse wave velocity; FMD, flow-mediated dilation; NMD, nitromediated dilation. *P* value refers to comparison vs. baseline.
*P less than 0.05.
**P less than 0.01.
***P less than 0.001.
****P less than 0.0001.

7.3 ± 1.4, $P < 0.05$; Fig. 2c). Baseline augmentation index did not differ between the two groups (Iva first 22.8 ± 12.6% vs. placebo first 20.2 ± 8.2%, $P = 0.8512$). Treatment with Iva increased augmentation index by 12.4% (Iva 24.3 ± 10.5 vs. placebo 21.3 ± 10.1, $P < 0.01$; Fig. 2d).

Aortic distensibility and circumferential cyclic strain

No difference in aortic distensibility and circumferential cyclic strain was detected between treatment groups at baseline. Treatment with Iva increased aortic distensibility by 33.3% compared with placebo (Iva 2.619 ± 1.249 10^{-3}/mmHg vs. placebo 1.743 ± 1.019 10^{-3}/mmHg, $P < 0.01$; Fig. 2e and Table 4). Circumferential cyclic strain was higher in patients treated with Iva (Iva 0.062 ± 0.027 vs. placebo 0.039 ± 0.018, $P < 0.0001$; Table 4).

DISCUSSION

This study investigated the impact of selective HRR induced by *If* channel inhibition with Iva on endothelial function and arterial stiffness in patients with stable CAD. The central novel findings are that sustained HRR over a period of 6 months restores brachial FMD, reduces cfPWV and increases central aortic distensibility. Moreover, HRR increases central aortic blood pressure and AIx75.

If channel inhibition by Iva reduced mean on-treatment HR by 15.7 bpm, an effect that is in line with data reported in the two major RCT's investigating Iva in CAD populations [13,29]. On placebo, HR was lower than measured at baseline most likely because of regression to the mean. Evidence for associations between HR reduction and vascular phenotypes has accumulated since the early 1980s in different animal models. Heart rate lowering decreased coronary atherosclerosis in monkeys after atrioventricular (AV) nodal damage. In dyslipidemic mice, selective pharmacological HRR with Iva restored endothelial function in different vascular beds [2,3,30–32]. However, to date despite broad evidence from animal studies, clinical data in humans investigating the effects of specific HRR on endothelial function is limited and not finally conclusive as a study in patients with diabetes showed that HRR by 20% (16 bpm) over a period of 4 weeks by atenolol but not by Iva improved endothelial function [33]. In patients with stable CAD, HRR of 10 bpm by Iva over an interval of 4 weeks did not affect endothelial function [34]. In contrast, a sustained HRR by 10% (6 bpm) induced by Iva over a period of 8 weeks improved FMD in patients with stable CAD undergoing coronary revascularization [35]. This finding is corroborated and expanded by data of the present study showing that HRR with Iva was stable over 6 months and resulted in a relevant increase in brachial FMD a surrogate of endothelial function that has been shown to be of prognostic relevance in individuals with CAD [36]. As all of the four studies constituted similar groups of high-risk patients treated with comparable background medical therapy and the achieved HRR is comparable across the studies, the central aspect that may explain those at first sight conflicting results is the duration of treatment and the time patients were exposed to lower HRs.

Several potential mechanisms that could account for HR-dependent restoration of endothelial function by Iva have been investigated. Among those, reduction of vascular oxidative stress, reduced expression of the angiotensin II type 1 (AT1) receptor, improved expression of eNOS and nitric oxide (NO) availability and downregulation of systemic and local inflammatory cytokine expression are the most prominent [3,15,31,32,37]. As potential HR-independent effects of Iva on the vasculature have been ruled out in those studies, HRR and the biomechanical consequences of HRR have been identified as the primary mechanism of action. Beyond the 'classical' factors perturbing endothelial function, vascular integrity may be affected by local hemodynamic characteristics induced by high HRs, such as

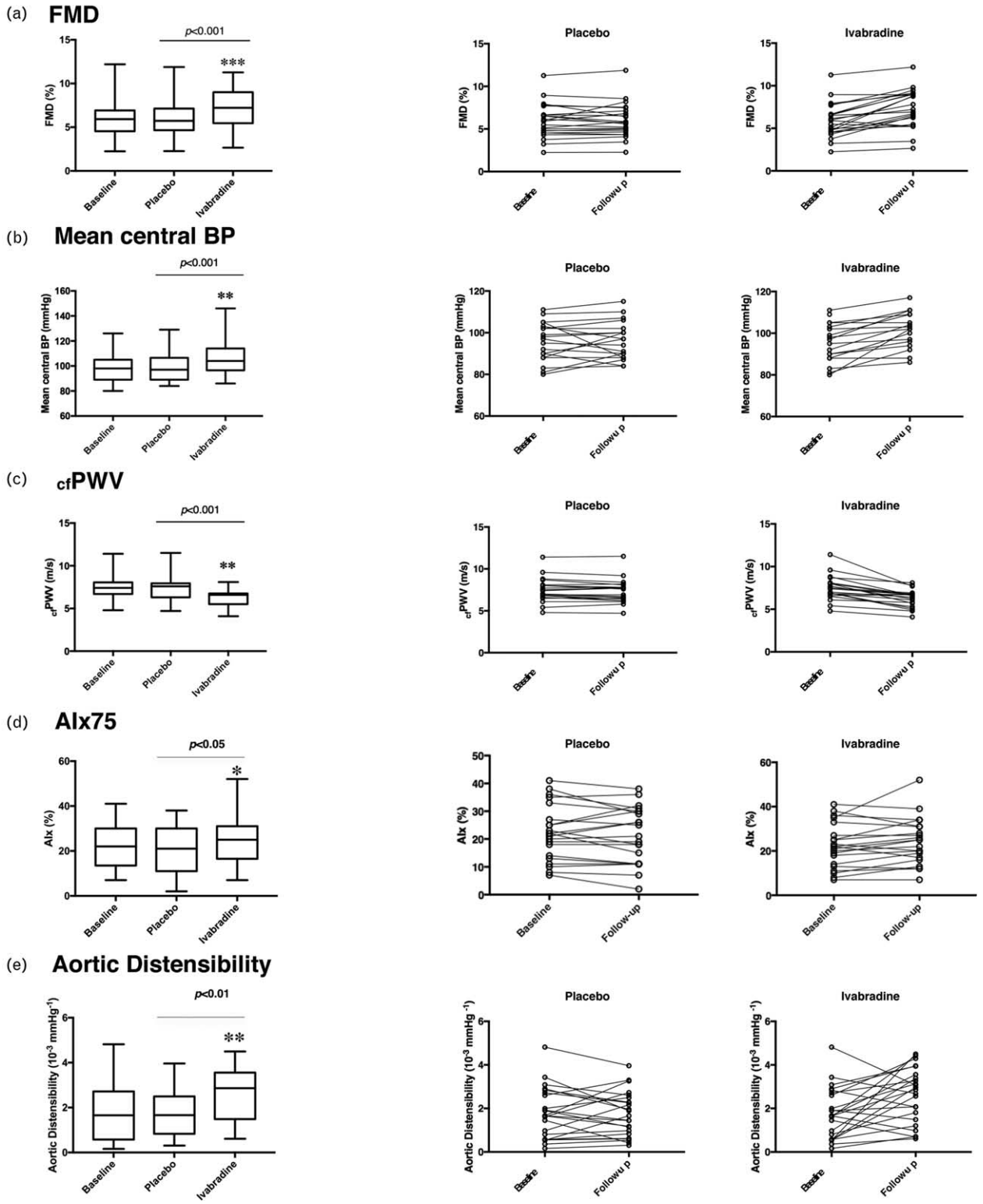

FIGURE 2 Effects of heart rate reduction with ivabradine on flow-mediated-dilation (a) mean central blood pressure (b), pulse wave velocity (carotid–femoral pulse wave velocity) (c), augmentation index (d) and aortic distensibility (e). *P less than 0.05, **P less than 0.01, ***P less than 0.001, ****P less than 0.0001. P value refers to comparison vs. placebo. augmentation index; FMD, flow-mediated dilation; PWV, pulse wave velocity.

endothelial shear stress or cyclic tensile stress [38,39]. As an integral component of pulsatile or cyclic stress (pulse pressure × HR), HR controls vascular mechanical properties and defines transmural force imposed on the vessel wall by pulsatile blood flow. Accelerated HR may promote low and oscillatory endothelial shear stress and enhance tensile stress and may synergistically promote atherogenesis [40]. First evidence for a potential link between shear stress and

HR originates from in-vitro studies in vascular endothelial cells suggest that shear waveform and frequency determine endothelial-cell gene expression [41]. However, as substantial evidence for a connection between shear stress and HR is sparse, one can only speculate that increased HR promotes disturbed (e.g. oscillatory) flow that may be 'redirected' to more laminar flow by HRR.

Arterial stiffness is a key feature of vascular aging, and one of the earliest detectable manifestations of adverse remodeling within the vessel wall [19]. Among various methods, measurement of cfPWV is the most validated method to noninvasively quantify arterial stiffness and serves as a strong predictor of adverse outcomes [42]. Whereas cfPWV is an average measure of overall arterial stiffness, evaluation of aortic distensibility and circumferential cyclic strain by MRI enables measurement of regional stiffness in the ascending aorta [43]. Both parameters were shown to be closely related to HR [22], [24]. In this study, we show that HRR with Iva reduces cfPWV and increases central aortic distensibility and circumferential cyclic strain. Thus, by combination of different methods, we provide robust evidence that HRR by Iva reduces arterial and central aortic stiffness.

Effects of HR on indices of arterial stiffness have been studied extensively in terms of pacing-induced tachycardia. In keeping with experimental data obtained in rats, it has been shown in humans that an increase in HR induced by cardiac pacing is associated with an increase in cfPWV [44–47]. Inversely, pharmacologically induced bradycardia has been investigated predominantly in animal models showing that selective HRR with Iva reduced arterial stiffness in terms of increased arterial distensibility [15,16,48]. To the best of our knowledge, this study is the first to examine effects of selective HRR with Iva on arterial stiffness in humans. An effect that may account for the close connection between HR and progression of arterial stiffening is fatigue failure of arterial elastin because of repetitive pulsatile mechanical stress. As accelerated HR decreases the period for arterial recoil and thereby augments pulsatile stress, this may contribute to thinning and fragmentation of arterial elastin and remodeling of the vessel wall [49,50]. Thus, reduction of pulsatility by HRR may represent a central mechanistic component of the destiffening effects of Iva.

Several mechanistic studies firmly established an inverse relationship between pacing induced acceleration of HR and AIx75, a ratio of central augmentation pressure and pulse pressure quantifying the contribution made by the reflected pressure wave to the ascending aortic pressure waveform [51–53]. Consistently an inverse relationship between HR and AIx75 and central aortic pressure was found in the CAFE (Conduit Artery Function Evaluation) study that compared effects of beta-blocker-based treatments with other BP-lowering strategies in hypertensive individuals [17]. Moreover, HRR with Iva for 6 months increased central aortic blood pressure in patients with stable CAD [18]. This phenomenon of impaired ventricular–vascular coupling can be attributed to a prolongation of the cardiac ejection time that is – because of lower HR – associated with enhanced augmentation of the central aortic SBP by the backward-traveling reflected pressure wave returning to the heart during systole instead of diastole [54,55]. In our study, HRR for 6 months by Iva led to a 12.4% increase in AIx75 and to a 7.8% increase of mean central aortic pressure. This finding corroborates the basic physiologic principle and extends knowledge as effects of HRR with Iva on AIx75 have not been tested until today. Our and other data [18] are apparently contrasting with findings showing that a short period (3 weeks) of HRR with Iva in individuals with stable CAD did not affect AIx75 and central aortic pressure [56]. As the patient population, concomitant beta-blocker therapy, dosing of Iva and methodology of pulse wave analysis were similar in our study the duration of treatment seemingly could account for differential observations. In view of the shorter treatment period of 3 weeks, one may speculate that in contrast to acute changes in HR because of cardiac pacing selective pharmacologic HRR induces effects on AIx75 and PP, which only manifest after extension of treatment duration. However, in view of longitudinal analyses [57] and RCT [10,29], at this point, the effect of the time difference (3 vs. 6 months) may be overrated and at least a potential time threshold remains elusive.

Thus, with regard to the available data, the relationship between HR, central hemodynamics and vascular function remains exceedingly complex and translation of those mechanistic findings into a clinical context is challenging. This is reflected by the controversial data on potential benefits of HR reduction across different patient populations. Focusing on the impact of HRR on central aortic pressure, there is broad evidence from interventional studies that HR and central aortic pressure share an inverse relationship. As central pressure was shown to be more predictive of cardiovascular outcomes than brachial pressure, the role of HRR in hypertension is discussed controversely. In the CAFE study, individuals randomized to atenolol had higher central systolic pressure than those given amlodipine, despite identical brachial pressures [17]. This effect observed with beta blockade may explain the potentially detrimental effect of beta blockade in hypertensive patients. In contrast, long-term observations over years (e.g. in hypertensive cohorts) consistently define HR as a relevant positive predictor of central hemodynamics and arterial stiffness [57,58]. Longitudinal analyses from the HARVEST study show that HR measured during sleep is positively associated with future AIx75 and central blood pressure in individuals with stage 1 hypertension [57]. Thus, short-term potentially maladaptive effects on central hemodynamics induced by HRR may be outweighed by beneficial effects on those parameters seen after longer periods of lower HR.

In our study, central pressure and AIx75 were significantly increased by Iva. In parallel, arterial stiffness was markedly decreased and endothelial function was improved. In vascular disease, for example, in age-dependent vascular dysfunction, endothelial dysfunction and arterial stiffness are closely interconnected [59]. Endothelial function determines arterial stiffness as the vascular endothelium affects the vascular mechanic properties by alterations in the arterial wall components. Moreover, endothelial dysfunction and arterial stiffness share the same underlying triggers [60]. Endothelial function was

independently and inversely associated with arterial stiffness in cross-sectional investigations in healthy individuals and in patients with CAD [61–63]. Thus, these beneficial effects might have counterbalanced the increase in central BP in well powered controlled clinical trials.

However, effects on central aortic pressure could also have been responsible for the lack of vascular outcome reduction in stable CAD seen in two major RCTs [13,29]. One could speculate that an increase of central blood pressure potentially abolishes the net benefit of HRR related to, for example, reduction of myocardial oxygen consumption. The double product and the time-tension index, both indices of myocardial oxygen consumption were not affected in a relevant manner by HRR with Iva [18]. In a clinical context, a pure negative chronotropic drug like Iva might, therefore, be beneficial in combination with antihypertensive drugs to effectively control blood pressure and to reduce central aortic pressure.

In conclusion, in this study, we show that sustained HRR over a period of 6 months in patients with stable CAD induced by Iva restored endothelial function and reduced arterial stiffness. HRR was associated with an increase in systolic central aortic blood pressure and AIx75. The former findings might have contributed to beneficial effects of HRR in heart failure or coronary artery disease with impaired left ventricular function at low blood pressures. The latter points towards the need for optimal BP control in vascular patients treated with Iva and require further in-depth investigation.

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Conflicts of interest

There are no conflicts of interest.

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