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OFFICIAL ANNOUNCEMENT

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Official Announcement of Physiological Diagnostic Criteria for Vascular Failure from the Japanese Society for Vascular Failure

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The Japanese Society for Vascular Failure has published a consensus statement on the physiological diagnostic criteria for vascular failure in *Hypertension*[1]. This was a long-desired effort of our academic society. Vascular failure was first proposed by Inoue and Node in 2006 as a highly integrated concept that includes a broad spectrum of vascular diseases, and was based on abnormalities of the vascular endothelium and medial layer components, as well as on metabolic abnormalities[2]. Vascular failure contributes to a wide range of vascular injuries from subclinical vascular damage to atherosclerotic arterial luminal narrowing, as well as circulatory dysfunction and structural abnormalities in the systemic and local vasculature. This indicates that vascular failure potentially plays an important role in the pathophysiology of various diseases and clinical conditions. Thus, it is likely that a larger population may be unexpectedly affected by vascular failure in routine clinical practice. However, the screening and diagnosis of vascular failure has not been appropriate, possibly due to its heterogeneous nature and lack of established diagnostic criteria. Based on these backgrounds, the present physiological diagnostic criteria were established as a clinical tool to systematically evaluate a subject's vascular function and/or diagnose vascular failure by the use of physiological tests.

To establish the criteria, we reviewed recent clinical evidence on the physiological tests to evaluate vascular endothelial function (flow-mediated vasodilation and reactive hyperemia-peripheral arterial tonometry) and integrated medial layer function by evaluation of arterial stiffness (pulse wave velocity and cardio-ankle vascular index). Then, we estimated two values for each test with the following goals: (i) to separate the normal and borderline zone associated with conventional cardiovascular risk factors, and (ii) to separate the borderline and abnormal zone associated with increased risk of cardiovascular events (**Figure 1**).

Hereafter, we need to disseminate these criteria widely

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Tests	X	Y
FMD (%)	7.0	4.0
RH-PAT	2.10	1.67
cfPWV (m/s)	Not estimated	10
baPWV (m/s)	14	18
CAVI	8.0	9.0

Figure 1. Diagnosis criteria for vascular failure and the cutoff values for each vascular test
A value of X indicates a cutoff value to separate the borderline from the normal range, and a value of Y indicates a cutoff value to separate the abnormal range from the borderline zone.
FMD: ≥ 7 for normal, ≥ 4.0 and <7.0 for borderline, and <4.0 for abnormal
RH-PAT: ≥ 2.10 for normal, ≥ 1.67 and <2.10 for borderline, and <1.67 for abnormal
cfPWV: ≥ 10 for abnormal
baPWV: <14 for normal, ≥ 14 and <18 for borderline, and ≥ 18 for abnormal
CAVI: <8 for normal, ≥ 8 and <9 for borderline, and ≥ 9 for abnormal
Modified from Tanaka A, et al. Hypertension 2018; 72: 1060-71.
FMD, flow-mediated vasodilation; RH-PAT, reactive hyperemia-peripheral arterial tonometry; cfPWV, carotid-femoral pulse wave velocity; baPWV, brachial-ankle pulse wave velocity; CAVI, cardio-ankle vascular index.

into clinical practice and to verify the clinical validity and efficacy of these criteria as appropriate surrogate markers for cardiovascular outcomes. In addition, we are now planning to establish the morphological and biochemical diagnostic criteria, so that we can comprehensively diagnose the vascular failure. Lastly, we hope that these criteria may help to identify possible patients with vascular failure in clinical practice and to implement pre-emptive and preventive medicine for cardiovascular disease.

Conflicts of Interest

K.K. received research funding from Omron Healthcare and Fukuda Denshi. H.I. received donation from Fukuda Denshi. Y.H. received consulting fees from Mitsubishi Tanabe Pharma Corporation related to this study, as well as honoraria and grants from Teijin, Boehringer Ingelheim, Merck, Sanofi, AstraZeneca, Kyowa Hakko Kirin, Takeda, Astellas, Daiichi Sankyo, Mochida, Nihon Kohden, Shionogi, Nippon Sigmax, Sanwa Kagaku Kenkyusho, Unex, and Kao, and honoraria from Radiometer, Omron, Sumitomo Dainippon, Otsuka, Torii, Kowa, Fujiyakuhin, Amgen Astellas, Nippon Shinyaku, Itamar Medical, Bayer, Eli Lilly, and Ono. The other authors declare no competing interests.

Acknowledgement

The physiological diagnostic criteria for vascular failure were developed by an expert committee in the Japan Society for Vascular Failure.

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ORIGINAL ARTICLE

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Disparity between EndoPAT measurement and brachial artery flow-mediated vasodilatation in hypertensive patients

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Abstract:

Background: EndoPAT measurement has been reported to be well correlated with brachial artery flow-mediated vasodilatation (FMD) in coronary artery disease. However, this relation is still controversial in hypertensive patients or normal subjects and the pathophysiological mechanisms of EndoPAT index is still not completely clarified. **Purpose:** The purpose of this study is to investigate the correlation of EndoPAT index with FMD and the physiological role of EndoPAT index in hypertension. **Methods:** To study this aim, we simultaneously measured EndoPAT index (RHI) and FMD by forearm occlusion technique, that is reported to be nitric oxide (NO) dependent, in 47 hypertensive patients without hypertensive complication (62±11 years old). BaPWV and augmentation index (AI@75bpm) by EndoPAT were also measured at the same time. **Results:** RHI did not correlate with FMD nor baPWV (r= 0.17, NS) while FMD also did not correlate with baPWV (r=0.08, NS). However, baPWV significantly correlated with AI@75bpm by EndoPAT (r=0.50, p<0.01). **In conclusion:** Augmentation index expressed as AI@75bpm by EndoPAT was associated with arterial stiffness measured by baPWV. However, there is no correlation between EndoPAT index of RHI and FMD in hypertensive patients, suggesting that the indexes reflect partially different vascular functions.

Key words:

FMD, EndoPAT2000, PWV, Hypertension

Introduction

Brachial artery (BA) flow-mediated vasodilatation (FMD) and nitroglycerin-induced dilation (NMD) in brachial artery (BA) is well known indices for evaluating endothelial function. However, especially FMD measurements are sometimes difficult and the reproducibility is limited despite well-established guideline[1-3]. Well-equipped UNEXEF18G system in Japan, that has recently been developed, as the semi-automatic image chasing measurement system, can precisely measure BA FMD[4]. In contrast to FMD, EndoPAT measurement has less bias for measurement and has excellent reproducibility[5,6]. In addition, hypertension is well known and common disorder to impair endothelial function[7]. For measuring the aortic stiffness caused by atherosclerosis, brachial-ankle pulse wave velocity (baPWV) an arterial augmentation index also has been utilized as the prognostic and diagnostic modality in the routine clinical practice[8]. The latter could be partly assessed by EndoPAT measurement.

In the previous report[9], EndoPAT measurement has been reported to be well correlated with BA FMD in coronary artery disease. However, this relation is still controversial in hypertensive patients or normal subjects and the pathophysiological measure of EndoPAT index is still not completely clarified.

Thus, to investigate the physiological role of EndoPAT index in hypertension, we simultaneously measured EndoPAT index (RHI) and FMD by forearm occlusion technique, that is reported to be nitric oxide (NO) dependent, in hypertensive patients without complication. In addition, baPWV and augmentation index (AI@75bpm) by EndoPAT were measured at the same time.

Methods

Study population and protocol

The study population consisted of 47 patients (27 men

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and 20 women; age, 63.2±10.6 years) who have visited our outpatient clinic of cardiology department in National Defense Medical College hospital under the diagnosis of hypertension without any complications. FMD and RHI have been approved by public insurance in Japan, and recommended in the guideline of Japanese Circulation Society for patient care[10]. The 37 subjects were also registered in FMD-J multi-center observational study[11], whereas the rest of 10 subjects were enrolled only for this investigation. Secondary hypertension such as primary aldosteronism and the patients with either renal artery stenosis or chronic renal failure had been worked out and excluded from the selection for study population. Additional exclusion criteria were as follows: 1) the presence of atrial fibrillation or diabetes mellitus; 2) advanced heart block; 3) any malignant disorders; 5) any other acute disorders; or 6) severe hypertensive patients who experienced hypertensive crisis. To use the patient's any information for this study, written informed consent was obtained from each patient and this clinical study was approved by institutional review board.

Blood pressure (BP), heart rate (HR) and body weight measurement

BP measurements were followed by the guideline of American Heart Association Scientific Statement[12]. The patients were asked to take away all clothing that covered the position of the cuff placement. And they were comfortably seated and the cuff on the upper arm was placed at the level of right atrium. The patients were instructed to relax, and at least 5 min passed before the BP measurement was carried out. All study patients had systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg when BP was obtained in the sitting position at outpatient clinics. BP was measured by Korotkoff's method using a sphygmomanometer. HR was directly obtained by stethoscope attached on the precordial region of the chest wall. Body weight was also measured at outpatient clinic.

Measurement of baPWV

The brachial-ankle PWV was measured using a volume plethysmographic apparatus (Form/ABI, Colin Co. Ltd., Komaki, Japan), in accordance with a previously described methodology[13,14]. In brief, occlusion cuffs, which were connected to both the plethysmographic and oscillometric sensors, were positioned around both the upper arms and ankles of the subjects lying in the supine position. The brachial and post-tibial arterial pressures were measured using the oscillometric sensor. The measurements were performed after the subjects had rested for at least 5 min in the supine position, in a temperature-controlled room (25℃) designed exclusively for this purpose.

Ultrasound FMD and RHI measurements in the brachial artery

All ultrasound studies were done in a temperature-controlled room (25℃) with the subject in a fasting, resting, and supine state from approximately 14:00 to 17:00. Heavy meals, including a high fat diet and caffeine-containing beverages, were prohibited beginning the night before the study. Patients were not allowed to have lunch on the day of ultrasound study. BP and HR were recorded from the left arm every 3 min with an automatic sphygmomanometer (Nihon Korin, BP-203, Tokyo, Japan) during the ultrasound procedure. Vasodilatation responses of the brachial artery were determined by the ultrasound technique using a semi-automatic device (EF18G; UNEX, Nagoya, Japan). Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 10-MHz linear array transducer. Then, a BP cuff was inflated to 50 mmHg above the systolic BP over the proximal portion of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diameter. The changes in the diastolic diameter were continuously recorded. Then, FMD was determined as the maximum change in diameter after cuff release normalized to the baseline diameter (% of baseline diameter). Calculation of these values by the EF18G in our laboratory showed that both the intra- and inter-observer variability (coefficient of variation) for repeated measures of diameter before and after reactive hyperemia in the brachial artery were < 3%[15,16].

Assessment of endothelial function by reactive hyperemia peripheral arterial tonometry (RHI measure) was simultaneously assessed by reactive hyperemia peripheral arterial tonometry using the EndoPAT2000 system (Itamar Medical, Caesarea, Israel) with FMD. As described previously[17], RHI was automatically calculated, and there is minimal inter-operator and intra-operator variability. Since FMD and RHI was measured simultaneously, RHI studies were performed when patients were in stable as described above. The RHI value that reflected the extent of reactive hyperemia was calculated as the ratio of the average pulse amplitude of EndoPAT2000 system signal over a 1-minute time interval starting 1.5 min after cuff deflation to the average pulse amplitude of EndoPAT2000 system signal of the 2.5-minute time period before cuff inflation (baseline). The RHI value was calculated by the EndoPAT2000 system. In addition, augmentation index (AI@75bpm) by EndoPAT was measured as arterial augmentation index. As mentioned earlier, previous studies have demonstrated that RHI has excellent reproducibility[17,18].

Statistical Analysis

Data are expressed as the mean ± SD. Even if the sample size was small, the histogram of each sample were not skewed (data not shown) so that we presumed each samples in this study were drawn from normally distributed data. Parametric statistical methods were subsequently utilized. Pearson product-moment correlation was performed between RHI, FMD, baPWV or augmentation index (AI@75bpm) by EndoPAT. Differences or statistical values were considered significant at $p<0.05$.

Table 1. Summarized clinical characteristics of the study population

	Study patients (n=47)
Age, years	63.2±10.6
Male/Female	27/20
Complications or comorbidities	
Hypercholesterolemia, %	11 (23%)
Hyperuricemia, %	4 (9%)
Combination treatment	
ARB , %	23 (48%)
CCB, %	38 (81%)
β blocker, %	5 (11%)
Statin, %	11 (23%)

Hypercholesterolemia, total cholesterol>220 mg/dl; Hyperuricemia, >7.0 ml/dl; ARB, Angiotensin II Receptor Blocker; CCB, calcium channel blocker; data are expressed as mean±SD or % in parenthesis

Table 2. Summary of body weight and ultrasound measurements of flow-mediated dilation in the brachial artery, brachial-ankle pulse wave velocity and indices in EndoPAT2000 system

Body weight, kg	64±16
Systolic BP, mmHg	136±12
Diastolic BP, mmHg	84±12
Heart rate, beats/min	69±11
Brachial artery diameter at baseline, mm	4.32±0.64
FMD, %	6.23±2.79
RHI	1.95±0.52
baPWV (cm/s)	1697±0.52
Augmentation index by EndoPAT	27±20

Data are expressed as mean±SD; BP, blood pressure; FMD, flow-mediated dilation; RHI, reactive hyperemic index; baPWV , brachial-ankle pulse wave velocity

Results

Patient Profile

The present study population consisted of 47 patients and the clinical characteristics are shown in **Table 1**. Mean age in study group was approximately 63 years. All patients had a clinically diagnosed hypertension. Approximately 23% of patients had hypercholesterolemia, and 4% had hyperuricemia. Combination medical treatment is also described in **Table 1**. As shown in the **Table 1**, 48% of patients have angiotensin II receptor blocker, 11% β blocker, and 23% statin treatment, and 81% patients had calcium channel blocker therapy. Hemodynamic and brachial artery diameter as well as the values of RHI, FMD, baPWV and augmentation index (AI@75bpm) by EndoPAT were listed in **Table 2**.

Correlation among RHI, FMD, and baPWV and augmentation index (AI@75bpm) by EndoPAT

Correlation between FMD and RHI, that between RHI and baPWV, that between FMD and baPWV and that between FMD and augmentation index (AI@75bpm) by EndoPAT were not significantly observed as shown in **Figure 1-4**. However, baPWV significantly correlated with augmentation index (AI@75bpm) by EndoPAT (**Figure 5**).

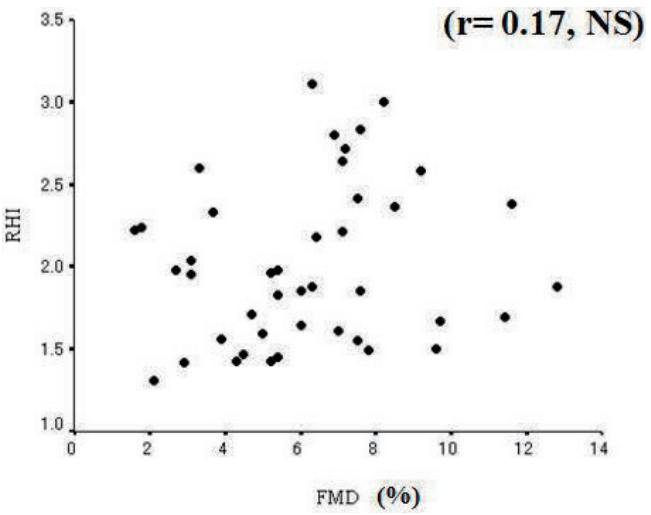

Figure 1. Relation between FMD and RHI
There was no significant correlation between them. FMD, flow-mediated vasodilatation; RHI, reactive hyperemic index assessed by EndoPAT.

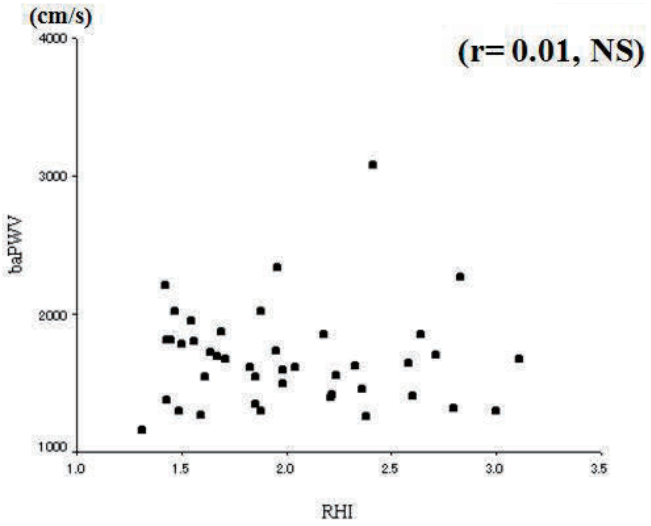

Figure 2. Relation between RHI and baPWV
There was no significant correlation between them. RHI, reactive hyperemic index assessed by EndoPAT; baPWV, brachial-ankle pulse wave velocity.

Discussion

The present study demonstrated that EndoPAT index of RHI might have different physiological property from FMD mainly reflecting NO metabolism, because the correlation between RHI and FMD was poor. Moreover, EndoPAT index of AI@75bpm was significantly correlated with baPWV in the hypertensive patients, suggesting AI@75bpm can be a useful index for arterial atherosclerotic changes as well as

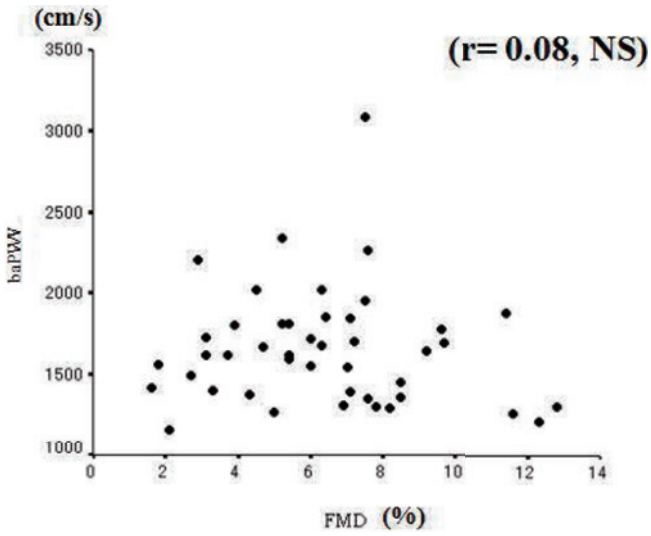

Figure 3. Relation between FMD and baPWV
There was no significant correlation between them. FMD, flow-mediated vasodilatation; baPWV, brachial-ankle pulse wave velocity.

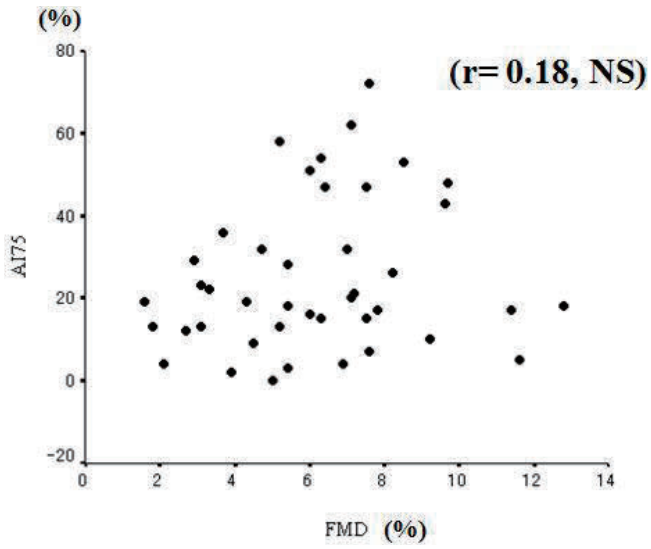

Figure 4. Relation between FMD and AI75
There was no significant correlation between them. FMD, flow-mediated vasodilatation; AI75, augmentation index (AI@75 bpm) by EndoPAT.

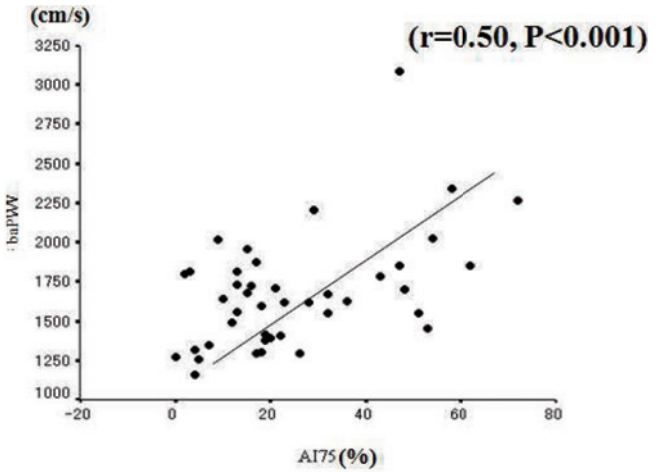

Figure 5. Relation between baPWV and AI75
There was significant correlation between them. baPWV, brachial-ankle pulse wave velocity; AI75, augmentation index (AI@75 bpm) by EndoPAT.

baPWV. EndoPAT index of RHI could reflect not only NO, but also endothelial derived prostaglandin I_2 (PGI_2) or endothelium-derived hyperpolarization factor (EDHF) in hypertensive patients. In contrast, AI@75bpm by EndoPAT could measure arterial elasticity and/or stiffness because baPWV reflects arterial elasticity while augmentation index expressed as AI@75bpm by EndoPAT can associate with arterial stiffness.

The relation among the indices measured in this hypertensive cohort was weak and these results support the above suggested conclusions. A shown in **Figure 1** to **Figure 3**, there were no correlation between RHI and FMD and between baPWV and either RHI or FMD. The correlation between AI@75bpm by EndoPAT and FMD was also not significant. Only significant correlation was observed between baPWV and AI@75bpm by EndoPAT. Since our hypertensive population was relatively homogeneous, these correlations are suggested to be significantly consistent findings for considering the relations among these indices in hypertensive patient. The results in this study are significant for these indices to be applied to the clinical practice.

The results in this study might not agree with the previous report[9,19], however, the study population is different between the studies. In the patients with coronary artery disease, RHI is reported to be significantly correlated with FMD. The reason why the concordant result was not obtained is not clarified by our study, however, the difference in patient population could be one of the explanation for this difference.

Even if the physiological backgrounds are different among the non-invasive vascular assessment indices of RHI by EndoPAT, FMD, baPWV, and augmentation index (expressed as AI@75bpm by EndoPAT), these parameters have separately been reported to predict the prognosis of cardiovascular diseases[20-23]. Any of these indices are thought to be useful in the clinical settings. However, some of these indices seem to be independent. The combination assessment such as RHI with FMD, baPWV or augmentation index could have a potential benefit for predicting untoward outcome of the patients with any significant atherosclerotic risk factors or the patients with overt cardiovascular disorders. Therefore, these evaluations are preserved as the further investigation of these indices.

Study Limitations

First, this study is cross sectional retrospective single center study and the numbers of study patients are small so that the power of the study is limited. In order to confirm our re-

sults and speculation, the results of FMD-J multi-center clinical trial[11] should be obtained and reviewed. Second, we performed endothelial function tests using simultaneous measures of FMD and RHI. However, we did not measure NMD, which is independent measure of endothelial function. To precisely measure endothelial function by FMD method, NMD should be measured. However, NMD measurement is out of our scope of purpose. In general, when endothelial function is preserved by FMD technique, NMD, that is the reflection of vascular smooth muscle function, is also well preserved so that NMD is presumed to be considered near normal in the present study.

Conflicts of Interest

The authors declare that we have no conflicts of interest.

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Is High Central BP but Normal Office Brachial BP a risk? —The ABC-J II Study—

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Abstract:

Background: Clinical significance of central blood pressure (BP) in treated hypertensives has not been established. We tested the hypothesis that subjects with high central systolic BP (CBP) but normal office brachial BP (OBP) have high cardiovascular risk profile. **Methods:** All of the subjects were participants enrolled in the Antihypertensives and Blood pressure of Central artery study in Japan (ABC-J) II study. Radial applanation tonometry (Omron 9000AI) was performed in 4077 subjects, and they were classified as; Group 1: high OBP (>140/90 mmHg) and high CBP (>130 mmHg); Group 2: high OBP and normal CBP; Group 3: normal OBP and high CBP; and Group 4 as both normal. Plasma brain natriuretic peptide (BNP) was used as a measure of cardiovascular load. **Results:** The mean age was 65.9 ± 11.2 yrs, 49.2% were females, and 25.7% had diabetes. In both genders, subjects with Group 3 were oldest, and beta-blocker was most frequently used, but body mass index (BMI), rate of diabetes, and heart rate were lowest among the four groups. In ANOVA, Group 3 tended to have higher BNP levels in both genders. In multivariate analysis after adjusting for significant covariates, BNP in Group 3 tended to be high level in females, but not in males. **Conclusion:** In treated hypertensives, higher central SBP was associated with higher BNP levels regardless of office brachial BP levels, especially in females. The results imply that high central SBP (>130 mmHg) can be used to detect high risk hypertensives with cardiovascular overload.

Key words:

Central blood pressure, Applanation tonometry, Brachial blood pressure, Brain natriuretic peptide, Cardiovascular load, Antihypertensives and Blood pressure of Central artery study in Japan (ABC-J) II study

Introduction

It has been shown that central blood pressure (BP) rather than peripheral BP was more useful to predict cardiovascular events. The Conduit Artery Function Evaluation (CAFE) sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) reported that central BP was more closely associated with cardiovascular prognosis than brachial BP[1]. In the Strong Heart study, central pulse pressure (PP) > or = 50 mmHg predicted adverse cardiovascular disease (CVD) outcomes[2]. In the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension (ACCOMPLISH) trial, the combination of calcium channel blockers (CCB) plus angiotensin-converting enzyme (ACE) inhibitor (ACE-I) was more effective than diuretics plus ACE-I combination because the reduction of central BP was larger in the former.

The Antihypertensives and Blood pressure of Central artery study in Japan (ABC-J) study consisting of 4000 treated hypertensive subjects started since 2007, and first report was published in 2010[3]. In this cross-sectional observation study, it was shown that vasodilatory antihypertensive agents lowered central BP independently of peripheral BP levels without evident class-specific differences, whereas non-vasodilators raised central BP. At present, the clinical application of central BP combined with brachial BP in treated

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hypertensives has not been established yet. In the present study, we sought to explore the clinical application of central BP, especially, focusing on treated hypertensives with normal office brachial BP but high central systolic BP (SBP).

Methods

Study design and subjects

This is a cross-sectional study, and subanalysis of the ABC-J II study, an expanded version of the original ABC-J study. Briefly, the ABC-J II study is a prospective observational study being conducted to evaluate the predictive values of central BP for cardiovascular events in Japanese treated hypertensive subjects. The protocol of the ABC-J II study has been registered on the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) website under the trial number UMIN000002966. All of the subjects in this study were treated hypertensive subjects enrolled in the ABC-J II study[3]. The hypertensive subjects had been under stable antihypertensive treatment for at least 3 months. The institutional review board of the Jichi Medical University School of Medicine and each participating institute approved this study, and written informed consent was obtained from all participants. Between January 2007 and May 2013, a total of 4,310 subjects were enrolled by 29 doctors at 27 institutions (13 primary practices, 3 hospital-based outpatient clinics, and 11 specialized university hospitals) throughout Japan.

The inclusion criteria were treated Japanese essential hypertensive subjects who met all the 3 criteria as 1) receiving a stable dosage of antihypertensive medication for at least 3 months; 2) subjects who have a data of radial tonometry data such as radial augmentation index (rAI) or central BP data; and the age 35 yrs or more. All the data were obtained from the medical records. Exclusion criteria were: (1) subjects with extremely abnormal BP [i.e., SBP 40 mmHg or diastolic BP (DBP) 20 mmHg higher or lower, respectively] compared to usual office BP or home BP during the examination; and (2) subjects with arrhythmia.

Hypertension was defined as office SBP >140 mmHg and/or diastolic BP (DBP) >90 mmHg, or the subjects being on antihypertensive medication[4]. Impaired fasting glucose was defined as fasting glucose levels ≥110 mg/dL, and impaired glucose tolerance was defined as glucose levels of ≥ 140 mg/dL at 2 h after a 75-g oral glucose tolerance test (OGTT)[5]. In the present study, diabetes mellitus (DM) was defined as one or more of the followings: self-report, the use of diabetes medication, fasting plasma glucose ≥126 mg/dL, or hemoglobin A1c (HbA1c) (NGSP) ≥6.5%[5,6]. The diagnosis of type 2 DM was based on current American Diabetes Association's criteria[5]. Dyslipidemia was defined as one or more of the following: self-report, total cholesterol level ≥ 240 mg/dL, triglycerides (TG) ≥150, high-density lipoprotein (HDL) <40 mg/dL, or a treatment for hyperlipidemia[7]. Heart failure (HF) was diagnosed by the Framingham criteria[8] as is widely accepted. Chronic kidney disease (CKD) was defined as the presence of overt proteinuria or estimated glomerular filtration rate (eGFR)<60 ml/min/1.73 m^2, or existing renal disease.

Blood pressure measurements

Office BP

Office BP was measured by physicians or nurses using sphygmomanometers in each institution based on the hypertension guideline. Arm circumference was measured and the appropriate cuff size was selected. We advised the subjects to take their morning medication as usual even on the days when they were visiting the clinics.

Blood and urine samples

Blood samples were drawn from the antecubital vein of the subjects. Blood and urine samples were collected in the morning in a fasting state during the study. Plasma/serum samples after separation and urine samples were stored at 4℃ in refrigerated containers and sent to a commercial laboratory (SRL Inc., Tokyo, Japan) within 24 hr. Plasma brain natriuretic peptide (BNP) was measured using high-sensitivity, noncompetitive radioimmunoassay (SHIONORIA® BNP, a radioimmunoassay (RIA) kit).

The estimated GFR (eGFR) was calculated using a validated equation based on the modified version of the Modification of Diet in Renal Disease (MDRD) study: eGFR (ml/min/1.73 m^2) = 194 × $Age^{-0.287}$ × $S\text{-}Cr^{-1.094}$ (if female × 0.739)[9]. Renal dysfunction was defined as eGFR<60 ml/min/1.73 m^2.

Assessments of the measures of target organ damage

Arterial wave reflection was assessed by rAI. The rAI was measured with a semi-automatic tonometry device (HEM-9000AI; Omron Healthcare Inc., Kyoto, Japan). The detailed method is described in a previous publication[10]. The HEM-9000AI was used to calculate the peripheral augmentation index as (P2-DBP)/(P1-DBP), taking P1 and P2 as the first and second inflection points on the radial pulse waveform[10]. In the present study, rAI was also expressed as rAI adjusted for heart rate 75 bpm. Central SBP (late systolic blood pressure in the radial artery, i.e. SBP2) was calculated by the equation described previously[10]. SBP2 measured by the HEM-9000AI was almost identical to central SBP measured by the SphygmoCor system[11]. The reproducibility of this device was confirmed in previous study[12]. In the present study, high central SBP was defined as >130 mmHg based on previous outcome study[13].

M-mode echocardiography, guided by a two-dimensional echocardiography, was performed based on the American Society of Echocardiography recommendations. End-diastolic left ventricular (LV) dimensions were used to calculate LV mass using an anatomically validated formula[14]. The LV mass index (LVMI) was calculated for each patient

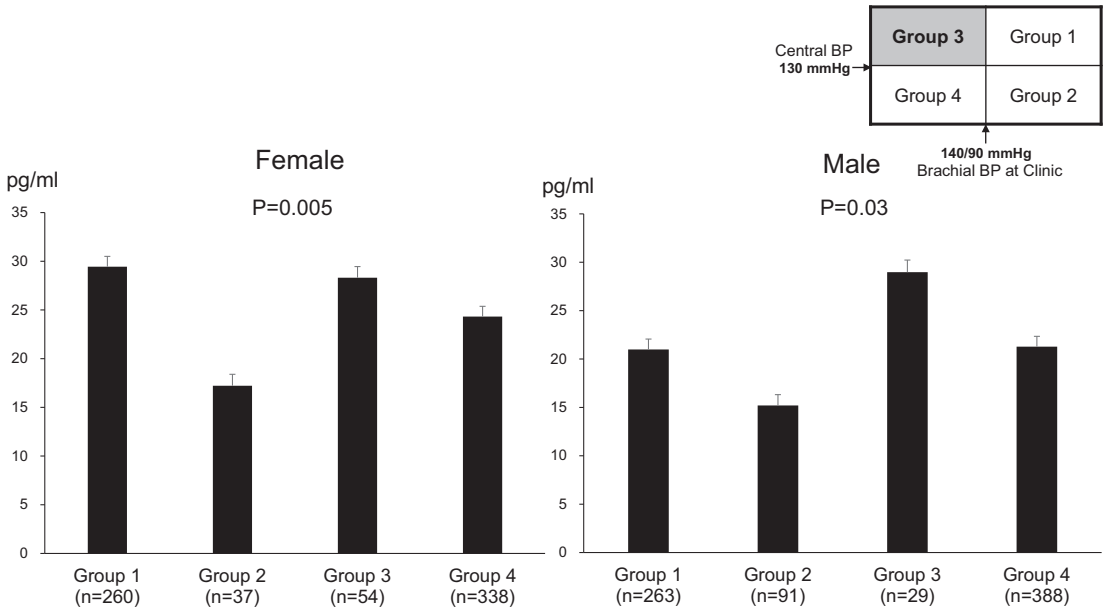

Figure 1. Comparisons of BNP in each group by ANOVA. Error bars shows standard errors. The definitions of each group are the same as Table 1.

by dividing LV mass by body surface area. The presence of LV hypertrophy (LVH) was defined by sex-specific criteria (LVMI ≥110 g/m^2 in women and ≥134 g/m^2 in men)[15].

Statistical analysis

All statistical analyses were carried out with the SPSS software package, version 19.0 (IBM-SPSS Inc., Armonk, NY). In the present study, all analyses were performed using the final dataset of the ABC-J II study. The analyses were performed separately by genders. The subjects were classified as; Group 1: high office BP (>140/90 mmHg) and high central SBP (>130 mmHg); Group 2: high office BP and normal central SBP; Group 3: normal office BP and high central SBP; and Group 4 as both normal BP. Because BNP data had a skewed distribution, and we transformed the data using the base-10 logarithm function. Data are shown as the mean ± standard deviation (SD) (continuous variables) or as percentages (categorical variables). Multivariate linear regression analysis was performed to analyze factors associated with central SBP. Factors associated with central SBP in the bivariate analysis or confirmed associating factors were entered as independent variables in this model. BNP levels among the four groups were compared using one way analysis of variance (ANOVA, **Figure 1**), and the general linear model adjusting for age, BMI, smoking, diabetes, CKD, HF history, and beta-blockers (**Figure 2**). Values of $p < 0.05$ were considered significant.

Results

There were 2007 females (67.1±10.8 years) and 2070 males (64.7±11.5 years); and 1046 (25.7%) subjects had diabetes.

Table 1 shows the characteristics of the subjects. In both males and females, the age tended to be higher in the Group 3 followed by the Group 1. BMI and the rate of diabetes tended to be higher in the Group 2 followed by Groups 1 and 4. Otherwise, there were no differences in clinical characteristics among the groups in both genders.

Table 2 shows the laboratory data among the four groups. In females, HbA1c was highest in the Group 2 among the 4 groups, and there were differences in total cholesterol and HDL-cholesterol levels. In males, AST, ALT, γ-GTP and LVH by echocardiography tended to be higher in the Group 2 than the others, however, there were no significant differences in the other items.

Table 3 shows the medications used in each group. In females, diuretics were more frequently used in the Group 4, and nitrates tended to be used more in the group 2, but no other differences were seen. In males, ARBs were more frequently used in the Groups 1 and 2, and beta-blockers were most frequently used in the Group 3 among the four groups.

Figure 1 shows the BNP levels among the four groups by ANOVA. In females, the Groups 1 and 3 had higher values of BNP followed by the Group 4 and 2. In males, Group 3 had highest value of BNP than the other three groups. When the age, BMI, smoking, DM, CKD, HF history, and the use of beta-blockers were further adjusted, the trend remained significant in women, whereas that of men became insignificant although the Group 3 had still relatively higher BNP value (**Figure 2**). When the analyses by using the cutoff value of BNP 40 pg/ml as the measure of high BNP level was performed with logistic regression analyses adjusting for age, BMI, smoking, diabetes, CKD, HF history and the use of beta-blockers, the trend was also significant in female, but not in men. However, the Group 3 had still relatively higher BNP value (**Suppl. Figure. 1**).

Table 4 shows the hemodynamic parameters by office

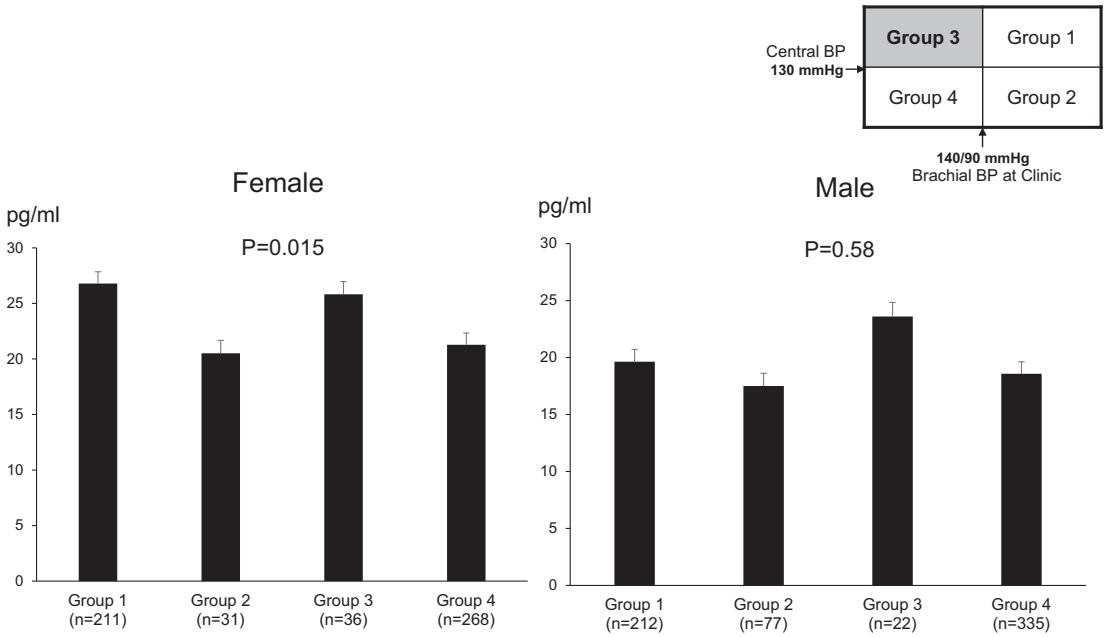

Figure 2. Comparisons of BNP in each group by the general linear model. BNP levels among the four groups adjusting for age, BMI, smoking, diabetes, CKD, history of HF and the use of beta-blockers. The definitions of each group are the same as Table 1.

Table 1. Characteristics of subjects

	Female					Male				
	Group 1	Group 2	Group 3	Group 4	P-value	Group 1	Group 2	Group 3	Group 4	P-value
N	782	76	160	989		692	201	84	1093	
Age, years	68±11.3	65.7±11.8	68.1±10.8	66.4±10.2	0.009	65.3±11.4	62.8±12.9	67.4±9.5	64.4±11.3	0.005
Body mass index, kg/m^2	24.1±3.9	25.7±4.7	23.5±3.5	24.2±3.9	0.001	24.5±3.1	25.3±3.7	24.1±2.9	24.5±3.3	0.006
†Smoking, %	5.4	3.4	7.8	5.0	0.61	27.8	28.7	15.3	22.3	0.02
‡Drinking, %	17.2	10.7	11.9	18.4	0.19	61.7	59	55.2	58.1	0.56
History of hypertension, yrs	2.9±1.0	3.1±1.0	2.9±1.1	3.0±1.0	0.50	2.9±1.1	3.0±1.1	3.0±1.0	3.0±1.0	0.32
Diabetes mellitus, %	21.7	39.5	18.8	26.2	0.001	27.0	35.5	17.3	27.9	0.01
Dyslipidemia, %	50.6	51.3	53.2	52.6	0.84	44.0	45.2	40.7	43.1	0.90
Hyperuricemia, %	7.3	6.6	7.1	8.6	0.73	19.4	21.8	28.4	23.1	0.14
Chronic kidney disease, %	5.6	3.9	3.9	5.0	0.76	7.5	7.1	7.4	7.8	0.99
Peripheral artery disease, %	4.7	6.6	3.2	4.3	0.70	4.4	7.1	4.9	4.4	0.45
Angina pectoris, %	8.8	11.8	9.1	8.6	0.83	12.1	15.7	11.1	14.4	0.39
Heart failure, %	2.2	1.3	1.9	3.1	0.52	1.3	2.5	3.7	3.1	0.10
Myocardial infarction, %	2.0	0	0.6	1.7	0.24	5.8	3.0	6.2	7.0	0.13
Cerebral infarction, %	3.3	6.6	1.3	2.8	0.19	3.7	4.1	3.7	4.5	0.85
Cerebral hemorrhage, %	0.1	0	0.6	0.4	0.53	0.4	0	0	0.4	0.55

Group1: high office brachial BP (>140/90mmHg) and high central SBP (>130mmHg); Group2: high office brachial BP and normal central SBP; Group3: normal office brachial BP and high central SBP; Group 4 as both normal BP.

Data are shown as the mean±standard deviation (SD) (continuous variables) or as percentages (categorical variables).

†smoking data was available 1533 in female and 1567 in male. ‡Drinking data was available 1507 in female and 1518 in male.

and central BP cutoff values. By definition, central BP was highest in the Group 1 followed by Group 3, but rAI, a marker of wave reflection, was highest, and HR was lowest in the Group 3 in both genders. Of note, PP amplification was also lowest in the Group 3 among the four groups.

Factors associated with central SBP by multiple linear regression analyses are shown in **Table 5**. Brachial SBP, BMI, diabetes, nitrate use, and beta-blockers were commonly associated factors with central SBP in both genders. BNP and alpha-blockers were associated with central BP only in females. When the hemodynamic parameters were analyzed by the presence of DM, central BP was significantly lower in the DM group than in the non-DM group (**Suppl Table 1**). When the subjects were further divided by DM plus genders, lower central BP was seen only in females but not in males (**Suppl Table 2**).

Discussion

In the present study, in treated hypertensives, high central

Table 2. Laboratory data in each group

	Female					Male				
	Group 1	Group 2	Group 3	Group 4	P-value	Group 1	Group 2	Group 3	Group 4	P-value
N	782	76	160	989		692	201	84	1093	
BUN, mg/dL	16.2±8.0	16.7±13.4	15.5±4.3	16.7±9.0	0.44	17.8±10.4	17.0±10.8	17.5±8.5	17.6±9.7	0.86
Serum creatinine, mg/dL	0.9±1.3	1.0±1.6	0.8±1.3	0.8±0.9	0.48	1.1±1.6	1.2±1.6	1.2±1.5	1.2±1.5	0.90
Uric acid, mg/dL	5.1±1.5	5.2±1.4	4.9±1.2	5.2±1.5	0.15	6.2±1.4	6.4±1.8	6.2±2.2	6.2±1.5	0.25
AST, U/L	24.6±19.1	23.1±7.9	24.3±9.5	23.5±9.8	0.62	25.3±11.4	29.6±18.5	23.1±6.6	25.2±13.4	0.002
ALT, U/L	21.2±17.3	22.5±15.0	22.0±13.9	21.2±13.4	0.90	26.4±17.9	35.7±31.0	21.7±9.6	26.3±18.5	<0.001
γ-GTP, U/L	28.4±25.5	35.4±41.2	28.2±26.5	30.1±30.3	0.53	57.8±68.4	69.1±66.9	38.9±23.6	53.2±52.7	0.02
Hemoglobin A1c (NGSP) %	5.6±0.8	6.1±1.3	5.5±0.6	5.6±0.9	<0.001	5.6±0.9	5.7±0.9	5.6±0.8	5.6±0.9	0.47
Total cholesterol, mg/dL	206±31	206±31	205±32	201±33	0.03	194±33	192±34	192±29	190±31	0.03
Triglycerides, mg/dL	121±101	127±68	118±51	116±61	0.49	141±94	145±80	137±102	141±91	0.90
HDL cholesterol, mg/dL	63±16	59±15	60±16	60±16	0.006	54±15	55±15	55±15	52±14	0.02
LDL cholesterol, mg/dL	121±32	123±34	119±29	118±30	0.31	114±30	114±34	110±27	113±29	0.67
LVH by UCG[1)], %	28.3	45.5	35.5	25.7	0.36	34.3	45.5	33.3	22.7	0.004
LVMI[2)], g/m^2	111±32	103±25	109±27	106±36	0.75	125±32	141±66	118±35	112±22	<0.001

BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltranspeptidase; NGSP, National Glycohemoglobin Standardization Program; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; LVH, left ventricular hypertrophy; UCG, echocardiography; LVMI, left ventricular mass index. Data are shown as mean±standard deviation (SD) (continuous variables) or as percentages (categorical variables) N=462 in females, 474 in males; 2) N=327 in females, 339 in males

Table 3. Cardiovascular medications in each group

	Female					Male				
	Group 1	Group 2	Group 3	Group 4	P-value	Group 1	Group 2	Group 3	Group 4	P-value
N	782	76	160	989		692	201	84	1093	
ARB, %	66.6	59.2	66.3	63.4	0.37	70.5	70.6	61.9	65	0.044
Diuretics, %	21.5	27.6	24.4	29.3	0.002	21.1	22.9	21.4	25.2	0.25
Calcium channel blockers, %	64.2	75	68.1	68.9	0.086	67.2	71.1	66.7	68.9	0.71
ACE inhibitors, %	7.5	7.9	5.0	8.2	0.57	8.8	7.5	11.9	10.8	0.31
Alpha-blockers, %	13.7	18.4	13.8	16.8	0.25	17.1	14.4	11.9	16.8	0.54
Beta-blockers, %	17.9	14.5	21.9	17.7	0.51	20.1	13.9	27.4	22.4	0.02
Nitrates, %	2.4	6.6	0.6	2.2	0.044	5.5	7.5	2.4	5.9	0.40
Other drugs, %	3.7	6.6	3.8	4.0	0.68	4.9	5.0	7.1	4.7	0.79

ARB, angiotensin receptor blocker; ACE, angiotensin converting enzyme

Table 4. Hemodynamic parameters in each group

	Female					Male				
	Group 1	Group 2	Group 3	Group 4	P-value	Group 1	Group 2	Group 3	Group 4	P-value
Brachial SBP, mmHg	155±12	144±9	137±2	123±10	<0.001	156±13	143±7	137±2	125±10	<0.001
Brachial DBP, mmHg	84±12	79±12	76±9	70±9	<0.001	88±13	83±11	78±8	72±10	<0.001
Brachial PP, mmHg	72±15	66±18	61±10	53±10	<0.001	68±14	60±15	59±8	53±10	<0.001
Central SBP, mmHg	149±13	125±6	133±2	114±11	<0.001	146±14	123±6	133±2	112±11	<0.001
Central PP, mmHg	65±15	47±13	57±10	44±10	<0.001	59±15	40±12	55±8	40±10	<0.001
PP amplification	1.12±0.11	1.43±0.3	1.07±0.05	1.22±0.17	<0.001	1.18±0.15	1.63±1.12	1.07±0.04	1.37±0.29	<0.001
AI, %	93.9±11	74.9±15.2	99.8±9.6	85.7±12.3	<0.001	87.5±12.1	67.2±12.1	97.3±7.6	76±13.3	<0.001
AI adjusted by HR 75, %	92.1±9.2	77.3±12.4	95.3±9.6	84.1±11	<0.001	84.5±10	68.9±11.5	91.2±7.5	73.8±11.6	<0.001
HR, bpm	71±11	81±14	65±9	71±11	<0.001	68±11	79±12	61±9	70±12	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; AI, augmentation index; HR, heart rate

BP was associated with higher level of BNP regardless of office BP levels in females. To the best of our knowledge, this is the first study to investigate the clinical relevance of the combination of high central BP and normal office BP in treated hypertensive subjects.

Central BP and cardiac overload

In the present study, central BP evaluated by radial appla-

Table 5. Factors associated with central SBP

	Female		Male	
	Beta	P-value	Beta	P-value
Brachial SBP, mmHg	0.934	<0.001	0.913	<0.001
Body mass index, kg/m^2	–0.047	<0.001	–0.063	<0.001
Diabetes	–0.08	<0.001	–0.031	0.047
log BNP	0.035	0.009	0.007	0.674
Nitrates use	–0.046	<0.001	–0.051	0.001
Beta-blockers use	0.052	<0.001	0.073	<0.001
Alpha-blockers use	–0.054	<0.001	–0.017	0.361

Multiple linear regression analyses

SBP, systolic blood pressure; BNP, brain natriuretic peptide

nation tonometry was associated with cardiac overload in treated hypertensive subjects. Central BP stands for cardiac afterload and has been shown to be associated with left ventricular hypertrophy (LVH)[16]. However, in the present study, the rate of LVH by echocardiography was not predominantly higher in the Group 3 than the others in both genders. Because the subjects in this study are all treated at least 3 months, subjects with inappropriately high BP (i.e. SBP> 180 mmHg) were rare.

We used BNP as a measure of cardiac overload. BNP has been shown to be important in identifying cardiovascular risk in hypertensives[17] and general populations[18,19]. BNP and NT-proBNP have been shown to be useful in identifying subjects with residual risk. The independent association between central BP and BNP would be important for risk stratification of subjects with cardiovascular risk factors, because the measurement of radial applanation tonometry takes only a few minutes and the result of this test can be obtained immediately. Multivariable adjustment including CKD and history of HF diminished the significant relationship between central BP and BNP in males (**Figure 2** and **suppl. Figure 1**), but did not change the results of females. However, it would be of value to measure central BP because complete assessment of cardiovascular risk factors is not always possible in clinical practice even in treated subjects, and the assessment of central BP in addition to brachial BP could be the clue for the further assessment of cardiovascular load.

Factors associated with high central BP

In the present study, factors associated with central BP were brachial SBP, BMI, DM, nitrate use, and beta-blockers in both genders, whereas, BNP and alpha-blockers in females. Brachial SBP, BNP and the use of beta-blockers were positively associated with central SBP, but the others were negatively associated with central SBP. Besides brachial SBP, which is predominantly associated with central SBP, obesity and DM have been reported to be negatively associated with central BP[20,21]. However, the other factors were positively associated with central SBP. Those subjects who are prescribed beta-blockers, alpha-blockers, and nitrates could have had some cardiovascular disease, which raise the levels of central SBP. Beta-blockers are shown to raise BNP levels in a population-based study[22]. Nevertheless, in our multivariate analyses, the use of beta-blockers was independently associated with central BP levels, which indicates that beta-blockers can raise BNP via the increased central hemodynamics.

Diabetes and central BP

In accordance with previous reports[21,23,24], when the hemodynamic parameters were analyzed by the presence of DM, central BP was significantly lower in the diabetes group than in the non-diabetes group. The lower central BP in diabetes was seen only in females but not in males (**Suppl Table 2**). This is in line with our recent study which showed that patients with diabetes had lower reflection wave than those without diabetes[25]. Increased proximal aortic stiffening in type 2 diabetes patients with less stiffened peripheral arteries, which is so-called "impedance mismatch", may both promote more penetration of pulsatile energy into the microcirculation of the brain and kidneys and reduce the reflections at systemic reflection sites. The reason for the gender difference is not clear, but it could be speculated that women tend to have less cardiovascular risk factors than males, and the effect of impedance mismatch in males could become more evident than in females.

Study limitations

There are some limitations in this study. First, the central BP was measured by radial applanation tonometry and there are some possibilities that inaccurate measurement results are contained. However, the device is semiautomatic and the method of this measurement was rigorously standardized in advance. Second, because the subjects in this study are all treated, it is revealed that some medications affect central BP levels, but it cannot be separated whether the association is purely the effect of medication or background cardiovascular disease. Third, in multivariable analyses, the significant relationship was seen only in females (**Figure 2** and **suppl. Figure 1**), but not in men. The exact reason cannot be clarified from this study, but it can be speculated that the weight of each covariates is relatively higher than high central BP in males. Insignificantly highest BNP levels in Group 3 in men may be due to the small number of subjects in this group (n=22). Finally, because of the cross-sectional nature of this study, the effects of beta-blockers on central hemodynamics and BNP levels are not clear. Beta-blockers may fail to reduce central BP in some individuals. This topic is somewhat complicated because the effect of beta-blockers on central hemodynamics and BNP levels depends on the generation of beta-blockers and comorbidity[26].

Perspectives

With regard to central BP, although a number of important findings that clarified the physiological mechanisms of arterial stiffness have been reported, the clinical applications

of these methods are still under way. Several studies have reported normal values or reference values of central BP[13,27,28], but even brachial BP values differ by age and gender[29]. In the present study, we tentatively set the normal value of central SBP as 130 mmHg based on data from Asia[13], we combined this with the normal office brachial BP value 140/90 mmHg, and then classified the subjects into four groups. As a result, the groups with high central SBP had higher values of BNP, a marker of higher cardiovascular burden. In light of the economic burden for patients, applanation tonometry is desirable because it takes only a few minutes to carry out, and there is no need to draw blood or perform expensive and time-consuming echocardiography. A prospective study is needed to confirm the clinical significance of this technique.

Conclusions

In treated hypertensives, higher central SBP was associated with higher BNP levels in women regardless of brachial BP levels at clinic. The results imply that high central SBP (>130 mmHg) can be used to detect high risk hypertensives with cardiovascular overload. Further prospective studies are needed to determine the clinical significance of central BP compared to brachial BP levels using hard outcomes.

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Contributors and details of the study investigators of the ABC-J II study are described also in the supplemental file (appendices).

Conflicts of Interest

The authors state that there are no conflicts of interest regarding this study.

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ORIGINAL ARTICLE

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Pretreatment with topiroxostat and irbesartan improves cardiac function after myocardial infarction in rats

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Abstract:

Background: Activation of angiotensin receptor type1 (AT1R) and xanthine oxidase (XO) generates reactive oxygen species (ROS), that causes cardiac dysfunction after myocardial infarction (MI). However, it remains unknown whether its inhibition could restore the cardiac function after MI. In the present study, we examined effects of irbesartan and topiroxostat on cardiac function after MI. **Methods and results:** We studied blood pressure and cardiac function in a rat myocardial infraction model using tail cuff system and echocardiography. Irbesartan and topiroxostat as well as vehicle were orally administered for 35 days to rats 7 days before MI induction. Neither irbesartan nor topiroxostat altered mean blood pressure and heart rate after MI. Treatment with either drugs significantly improved cardiac function after MI. The potency of topiroxostat to restore the cardiac function was approximately half of that of irbesartan. **Conclusions:** A non-purine XO inhibitor, topiroxostat improved cardiac function after MI, suggesting that like irbesartan, topiroxostat may be a promising drug to treat congestive heart failure after MI.

Key words:

Topiroxostat, Irbesartan, Xanthine oxidase, Myocardial infarction

Introduction

Myocardial infarction (MI) has the highest mortality rate and contributes to the development and progression of heart failure (HF)[1)]. HF remains a major health problem worldwide, and there is an urgent need to develop a new therapeutic strategy[2)].

Several studies have reported that reactive oxygen species (ROS) play important roles in the pathophysiology of cardiac remodeling after MI[3)]. In vitro, exposure of cardiomyocytes to ROS generated by xanthine oxidase (XO), a potent enzymatic source of ROS, has been shown to promote cardiac hypertrophy and dysfunction[4)]. In addition, ROS[5)] caused mitochondrial injury by inhibiting the activity of various respiratory-chain enzymes, leading to a decrease in myocardial ATP production and altered glycolipid metabolism. Therefore, inhibition of XO might attenuate ROS production and protect cardiac mitochondria from oxidative damage, thereby attenuating cardiac function in congestive heart failure (CHF). Allopurinol, an authentic XO inhibitor, is used worldwide for the treatment of hyperuricemia. Several studies using both animals and humans have shown that allopurinol improves cardiac dysfunction, mechano-energetic coupling and tolerance to exercise with after MI, cardiomyopathy and HF by decreasing cardiac ROS production and increasing cardiac energy (ATP)[6-8)]. However, attention

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should be paid to doses of allopurinol because it has various side effects, such as allergies and liver dysfunction in patients with impaired renal function. Topiroxostat, a non-purine selective XO inhibitor, is a recently developed more potent inhibitor of XO than allopurinol, without any significant inhibitory effects on other enzymes such as aldehyde oxidase as well as purines and pyrimidine enzymes. Several studies revealed that topiroxostat protects kidney cells from apoptosis owing to its antioxidant activity in vivo[9,10]. However, the effects of topiroxostat on cardiac function after MI remains unelucidated.

Angiotensin II (Ang II) also plays a key role in the pathogenesis of myocardial repair/remodeling after MI. Ang II leads to vasoconstriction, cell growth and positive inotropic action by increasing the secretion of aldosterone through the activation of Ang II type I (AT1) receptor. AT1 signal plays a pivotal role in the progression of post-infarct left ventricular (LV) remodeling associated with CHF. Experimental studies have also shown that inhibition of RAS by AT1 blockers shows beneficial effects on rat HF or MI models[11]. Moreover, these results have been confirmed in human HF. In a clinical setting, inhibition of RAS by AT1 receptor blockers or angiotensin-converting enzyme inhibitors (ACEIs) is a standard therapy for patients with MI and CHF. Irbesartan is one of the AT1 receptor blockers, which has non-hemodynamic cardiovascular and renal protective effects. Berthonneche et al showed that irbesartan improved cardiac function and remodeling mediated by TNF-α inhibition after MI in rats[12].

In the present study, to evaluate the effects of pretreatment with topiroxostat and irbesartan prior to the induction of MI, we studied chronic effects of topiroxostat on the cardiac function and remodeling after MI and compared them to those of irbesartan.

Methods

Animals and Experimental groups

Male syngeneic Lewis rats (body weight 200 to 250 g, 8 weeks old) were obtained from Japan SLC, Inc (Hamamatsu, Japan). The experimental protocols were approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, Tottori University.

Irbesartan and topiroxostat as well as vehicle were orally administered to rats 7 days before creation of MI. Rats were randomly allocated into four groups: (1) Sham group (n=5) (2) MI + vehicle group (n=4) (3) MI + topiroxostat group (n =5), and (4) MI + irbesartan group (n=5). Topiroxostat (0.5 mg/kg)[13] (in MI + topiroxostat group), irbesartan (30 mg/kg)[12] (in MI + irbesartan group) or vehicle (0.5 mL) (in sham group and MI + control group) were administered to rats once per day from day -7 to day 35 by gavage using a stomach tube.

Induction of myocardial infarction

Rats were anaesthetized by inhalation of isoflurane (3-5%; DS Pharma Animal Health, Osaka, Japan), intubated and mechanically ventilated via tracheal cannula connected to a constant volume ventilator (60 cycles/min, 1 mL/100 g). Left thoracotomy and pericardiotomy were performed, and the left main anterior coronary artery was completely ligated 1-2 mm from its origin with a 6-0 polypropylene suture[12] on day 0. Coronary occlusion was verified by the rapid occurrence of akinesia and discoloration in the area at risk.

In vivo measurement of blood pressure and heart rate

Systolic and diastolic blood pressure (BP) and heart rate (HR) were measured by a tail cuff system (BP-98A, Softron, Tokyo, Japan) on the day -7 (before MI operation and just before drug administration), day 0 (just before MI operation), days 7, 21 and 35. The mean arterial pressure (MAP) was calculated from measured systolic and diastolic BP.

Echocardiographic analysis

Cardiac function and LV morphology were evaluated with transthoracic echocardiography using the LOGIQ P5 system with a 12-MHz probe (12 L, GE Healthcare, Fairfield, CT). Echocardiography was performed under anesthesia with isoflurane (3-5%) on days 0, 7, 21 and 35. We used the images of mid-papillary short-axis (SAX) views of the LV for analysis of LV end diastolic dimension (LVEDD), LV end systolic dimension (LVESD), anterior wall thickness (AWT), and fractional shortening (FS). All measurements were made in triplicate and averaged by two independent experienced examiners in a blinded fashion.

Statistical Analysis

Comparisons of the cardiac function, MAP and HR among multiple groups were determined by one-way ANOVA with the Tukey-Kramer test. All data are expressed as the mean ± S.E.M.; $P<0.05$ was considered statistically significant. Comparisons within a group were made by repeat measures one-way ANOVA followed by the Bonferroni multiple comparison post test analysis when the global test was significant. Two-way ANOVA was used to compare effects of topiroxostat, irbesartan and vehicle, followed by Bonferroni post tests. Unpaired t-test was performed for comparison between groups.

Results

Effects of topiroxostat and irbesartan on MAP and HR

There were no changes in MAP either in the Sham or the MI + topiroxostat group during the entire period. In the MI + vehicle group, MAP trended to decrease on day 7 compared to that on day -7. In the MI+ Irbesartan group, MAP

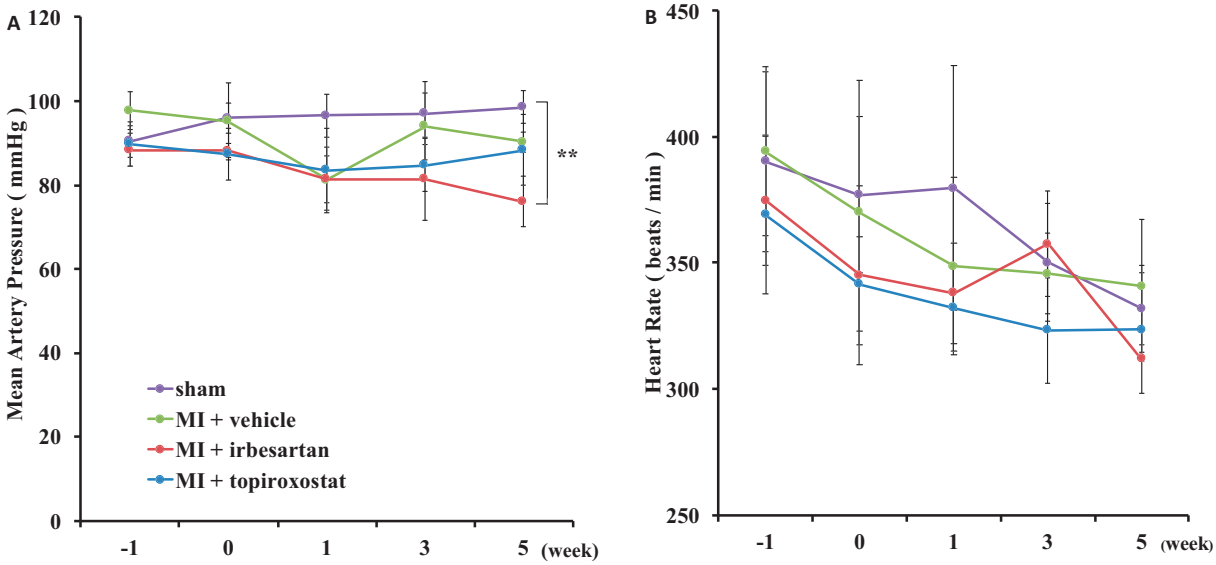

Figure 1. Effects of topiroxostat and irbesartan on the blood pressure before and after myocardial infarction.
Red: irbesartan, blue: topiroxostat. Green; vehicle, purple: sham
*: p<0.05 vs sham
**: p<0.01 vs sham

on day 35 significantly decreased compared to that in the Sham group (**Figure 1A**). There was no significant difference in changes in HR among all groups during the entire period (**Figure 1B**).

Effects of topiroxostat and irbesartan on cardiac changes

Both LVEDD and LVESD significantly increased after MI, while AWT and FS significantly decreased in the MI + vehicle, MI + irbesartan and MI + topiroxostat groups compared to those in the Sham group (**Figure 2**). In the MI + irbesartan group, LVEDD significant decreased compared to that in the MI + vehicle group (on day 7 and day 35) and MI + topiroxostat group (on day 35) (**Figure 2A**). In the MI + irbesartan group, LVESD also significantly decreased compared to that in the MI + vehicle group (from day 7 to day 35), and the MI + topiroxostat group (on day 7 and day 35) (**Figure 2B**). There was no significant difference in AWT after MI among the 3 groups (MI + vehicle, irbesartan and topiroxostat groups) (**Figure 2C**).

FS significantly increased in the MI + irbesartan group, compared to that in the MI + vehicle group on days 21 and 35. In the MI + topiroxostat group, FS also significantly increased compared to that in the MI + vehicle group at the same time points (**Figure 1D and F**). However, FS in the MI + topiroxostat group was significantly lower than that in the MI + irbesartan group (FS on day 35; 39.1% in the sham group, 13.8% in the MI + vehicle group, 16.8% in the MI + topiroxostat group, 21.6% in the MI + irbesartan group).

Discussion

In this study, we demonstrated the effects of an XO inhibitor topiroxostat and an AT1 receptor blocker irbesartan started 7 days before MI (day -7), on cardiac function and remodeling up to day 35 (42 days observation period). To our knowledge, this study is the first report that directly compared effects of two drugs on cardiac function and remodeling after MI.

The most prominent finding is that topiroxostat significantly improved LV function (FS), although it did not show protective effects on LV remodeling (LVEDD and LVESD). The precise mechanisms on the protective action of topiroxostat on cardiac function without protective action on remodeling remain unknown.

In vitro studies using isolated hearts have shown that progressive development of HF is associated with increased myocardial XO levels, resulting in an increase in cardiac ROS[13,14]. It has been reported that XO-derived ROS could interfere with nitric oxide regulation of myocardial energetics[15] and depressed myocardial excitation-contraction coupling[16]. Allopurinol has been reported to decrease myocardial oxygen consumption and increase cardiac contractility and mechanical efficiency[17]. In the present study, topiroxostat significantly increased cardiac contractility (estimated by FS after MI), indicating that reduction in ROS production by topiroxostat may restore nitric oxide regulation of myocardial energetics.

In contrast, topiroxostat could not ameliorate cardiac remodeling after MI. We recently showed that topiroxostat (3 μmol/L) prevented LV dysfunction and facilitated recovery from arrhythmias using an ischemia-reperfusion (I/R) model of rat heart. Topiroxostat inhibited XO activity to a much

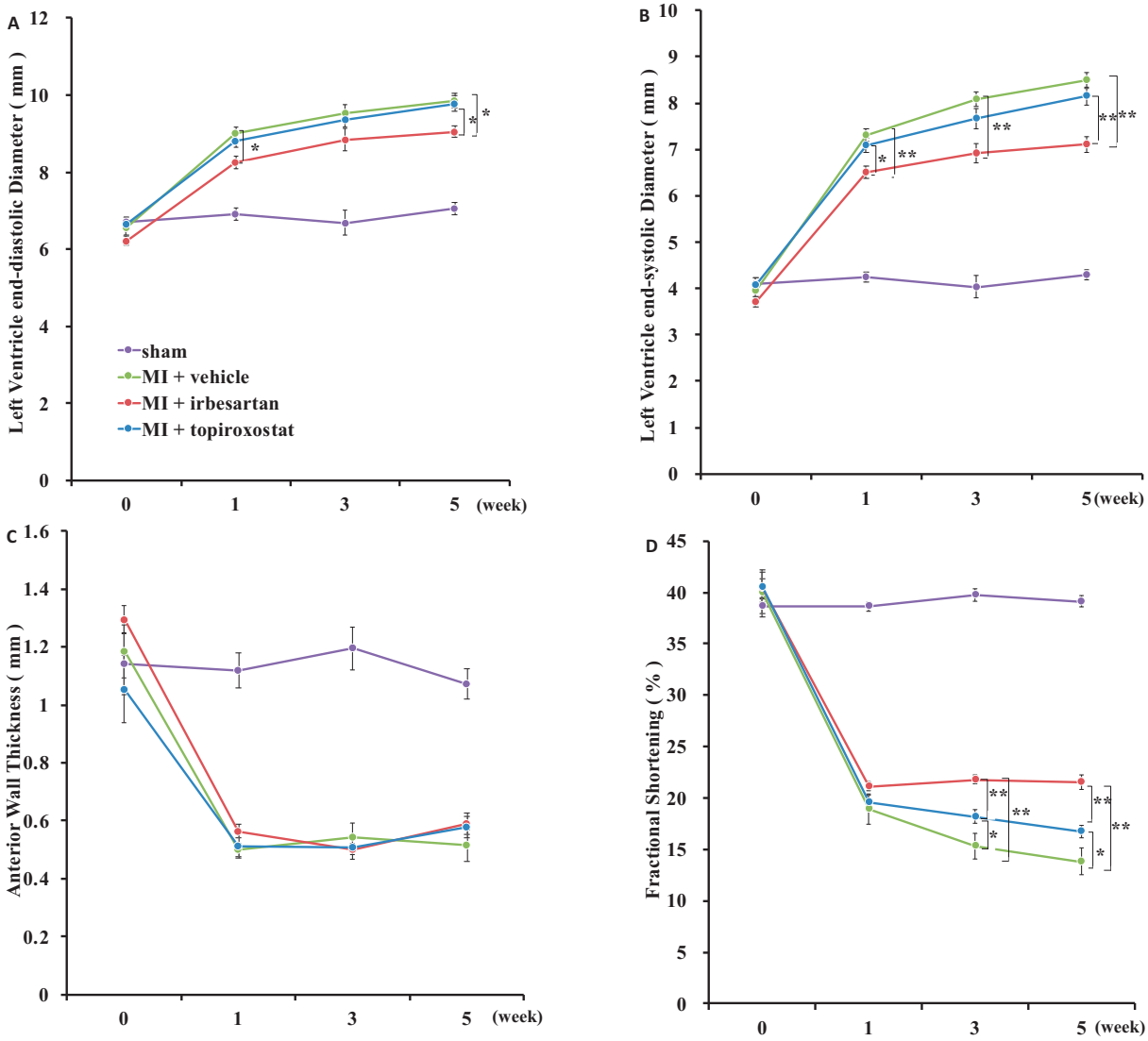

Figure 2. Effects of topiroxostat and irbesartan on the cardiac function before and after myocardial infarction.
Panel A: Effects of topiroxostat and irbesartan on FS before and after myocardial infarction. Note that topiroxostat and irbesartan significantly improved FS.
Panel B: Effects of topiroxostat and irbesartan on LVEDD before and after myocardial infarction. Note that irbesartan significantly improved LVEDD.
Panel C: Effects of topiroxostat and irbesartan on LVESD before and after myocardial infarction. Note that irbesartan significantly improved LVESD.
Panel D: Effects of topiroxostat and irbesartan on AWT before and after myocardial infarction. Note that both agents did not improve AWT.
Red: irbesartan, blue: topiroxostat. Green; vehicle, purple: sham
LVEDD: Left ventricular end diastolic dimension, LVESD: Left ventricular end systolic dimension, AWT: anterior wall thickness, FS: fractional shortening
*: p<0.05, **: p<0.01

greater extent than allopurinol[18]. Sugiyama et al. indicated that the minimum and maximum plasma concentrations of topiroxostat were estimated to be 0.93 and 7.1 μmol/L, respectively, which are reached by oral administration of topiroxostat at 20 and 180 mg/day[19]. Topiroxostat concentration in the I/R model at 3 μmol/L was within its clinical concentration. In this study, we orally administered of topiroxostat at 0.5 mg/kg/day in rats. We chose this dose because there was a possibility of nephropathy at 1 mg/kg/day[20]; thus, we prioritized safety and reduced the dosage considerably. The dosage of 0.5 mg/kg/day was very low compared to the clinical dosage (2.5-3 mg/kg/day). Thus, one of the possible reasons that topiroxostat did not show a protective effect on cardiac remodeling in this study may be under-dosage of topiroxostat. In addition, topiroxostat has reported to show mechanism-based and structure-based inhibition of XO without any inhibitory actions on other enzymes. In the MI model, the mechanisms of LV remodeling include multiple factors, such as TNF-α, NF-κB, and activator protein-1 (AP-1) not only ROS. The different mechanisms between I/R

model and MI model was one of the possible reasons why topiroxostat showed the protective effect on cardiac function without any protective action in the MI model.

Irbesartan prevented LV remodeling starting from an early phase (day 7) to the chronic phase (day 35) and improved LV function in the chronic phase. On day 35, MAP was lower in the irbesartan group than in the sham group. It is generally accepted that Ang II promotes cardiac remodeling via AT1 receptors, which increase in the heart after MI. Ang II activates various transcription factors, such as TNF-α, NF-κB and AP-1. Irbesartan is a high selective and long-acting AT1 receptor blocker. Berthonneche et al showed that administration of irbesartan in the early phase (7 days after MI) improved cardiac function and cardiac geometry mediated by inhibition of myocardial TNF-α generation in an MI rat model[12]. Watanabe et al showed that irbesartan exerted antifibrotic and anti-inflammatory effects by inhibiting the activation of NF-κB, AP-1 and NOX4 28 days after MI induction simulating cardiorenal syndrome in a rat model[21]. In addition, Schafer et al indicated that irbesartan restored aortic eNOS expression and reduced aortic superoxide formation in CHF 10 weeks after MI induction in rats[22]. We have also reported that irbesartan chronically suppressed LV remodeling after MI, which may be related to reduced TNF-α, NF-κB, AP-1 and MAP[21].

It is interesting to compare the potency of topiroxostat to protect cardiac function to that of the AT1 receptor blocker, irbesartan, a standard treatment for CHF. Although the cardioprotective effect of topiroxostat on the MI heart was weaker than that of irbesartan, it significantly improved cardiac function compare to the vehicle group. Since its mechanism on improving cardiac function is different from that of irbesartan, a combination of topiroxostat and irbesartan may accentuate their protective action on cardiac function after MI.

Our study has several limitations. First, it is unclear whether the dosages of topiroxostat and irbesartan were appropriate and equipotent. There are not many reports regarding appropriate doses of topiroxostat in a rat model. As mentioned above, we prioritized safety and reduced the dosage considerably. Topiroxostat dosage of 0.5 mg/kg/day may be too low. More studies are required about topiroxostat dosage. We used irbesartan at 30 mg/kg/day[12], which was within the no-observed-adverse-effect level and much higher than the clinical dosages (3-3.5 mg/kg/day). Second, we studied MAP, HR and a few echocardiographic parameters, but did not study changes in cytokine levels, hemodynamics, oxidative stress and uric acid level. Further studies are needed to clarify the detailed mechanisms. Third, we did not measure the marker of oxidative stress such as TBARS to evaluate the effect of topiroxostat and irbesartan on oxidative stress. Fourth, in the present study, we examined whether pretreatment with topiroxostat and irbesartan prior to MI could improve cardiac dysfunction after MI. However, we did not evaluate why there was no significant difference in the size of MI among groups because of experimental limitations.

In conclusion, a non-purine XO inhibitor, topiroxostat, improved cardiac function after MI. Although it was shown that topiroxostat had different mechanisms for the cardiac protective effect compared to irbesartan and it exerted lesser cardiac protective effects than irbesartan, topiroxostat will be a potential drug for improving cardiac functions after MI.

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Conflicts of Interest

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ORIGINAL ARTICLE

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Arginine deficiency measured by global arginine bioavailability ratio in patients with acute coronary syndrome

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Abstract:

Background: L-arginine and its related metabolites are associated with arginine bioavailability and subsequent nitric oxide production. The global L-arginine bioavailability ratio (GABR), defined as the ratio of the level of L-arginine to the sum of the levels of its major metabolites (L-arginine/[L-citrulline + L-ornithine]), has been reported as an index of arginine bioavailability. GABR in acute coronary syndrome (ACS) has not been fully investigated. **Methods and results:** The serum levels of L-arginine, L-citrulline, L-ornithine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) were assessed in 134 patients who underwent coronary angiography. The patients were classified into the following three groups based on clinical presentation, electrocardiogram, and coronary angiogram: stable patients without coronary artery disease (CAD (−), n = 38), stable patients with CAD (CAD (+), n = 56), and patients with ACS (n = 40). The ACS patients included 13 with acute myocardial infarction and 27 with unstable angina pectoris. L-arginine and L-ornithine levels were not significantly different among the three groups, whereas L-citrulline levels were significantly increased in ACS patients (CAD (−): 135 ± 62, CAD (+): 148 ± 68, ACS: 174 ± 79 μmol/L; p = 0.043), resulting in a significant decrease in GABR (CAD (−): 0.51 ± 0.19, CAD (+): 0.49 ± 0.17, ACS: 0.39 ± 0.12; p = 0.003). **Conclusion:** Increased serum citrulline and decreased GABR were observed in patients with ACS, suggesting the presence of relative arginine deficiency in ACS.

Key words:

Global arginine bioavailability ratio, GABR, Acute coronary syndrome, L-arginine, L-ctrulline

Introduction

Nitric oxide (NO) is an important regulator of vascular tone and homeostasis, and in normal arteries, it plays important roles in vasodilation and in inhibition of platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation. Decreased bioavailability of NO in endothelial cells causes endothelial dysfunction, resulting in hypertension, atherosclerosis, and cardiovascular disease[1]. Because NO is produced by nitric oxide synthase (NOS) using L-arginine as the sole substrate, decreased bioavailability of L-arginine causes decreased NO production and subsequent endothelial dysfunction[2].

L-arginine is obtained from endogenous synthesis and degradation of body protein in addition to the diet, and so serum L-arginine concentration is generally maintained at a level high enough for NO production[3,4]. However, despite having normal serum levels of L-arginine, patients with hypertension, hyperlipidemia, or diabetes mellitus can have decreased L-arginine bioavailability and subsequent endothelial dysfunction, which can be alleviated by exogenously supplied L-arginine. This phenomenon is called the "arginine paradox"[5].

One of the causes of the arginine paradox may be the elevated level of asymmetric dimethylarginine (ADMA)[5]. ADMA is an endogenous inhibitor of NOS and is generated from methylation of L-arginine residues of intracellular proteins by protein arginine N-methyltransferases and subsequent breakdown of the proteins[6]. ADMA competes with L-arginine as a substrate of NOS, resulting in decreased L-arginine bioavailability and endothelial dysfunction. Another cause of the arginine paradox may be consumption of L-

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arginine by increased activity of arginase[7,8]. L-arginine is converted to NO and L-citrulline by NOS and to L-ornithine and urea by arginase. Because L-arginine is the common substrate for NOS and arginase, increased activity of arginase in endothelial cells may decrease the intracellular L-arginine level, limiting bioavailability of L-arginine and causing endothelial dysfunction.

The metabolites of L-arginine also affect arginine bioavailability. L-ornithine and L-arginine share the same plasma membrane transporter; therefore, in the setting of increased serum L-ornithine levels, L-ornithine competes for the transporter with L-arginine and limits the uptake of L-arginine by endothelial cells, resulting in decreased L-arginine bioavailability[4].

The global L-arginine bioavailability ratio (GABR), defined as the ratio of the level of L-arginine to the sum of its major metabolite levels (L-arginine/[L-citrulline + L-ornithine]), has been reported as an index of arginine bioavailability in various clinical settings[9-14], including stable coronary artery disease (CAD)[15,16]. In acute coronary syndrome (ACS), GABR was reported to be lower in patients with cardiogenic shock due to acute myocardial infarction than in patients with stable CAD[17]. However, cardiogenic shock is reported to be associated with increased NO production[18], which may alter GABR, so GABR in ACS has not been fully studied.

In this study, we measured serum levels of L-arginine, L-citrulline, and L-ornithine and then compared these values and GABR between patients with ACS and stable patients with or without CAD who underwent elective coronary angiography. Because ADMA can influence GABR through inhibition of NOS, ADMA concentration was simultaneously measured.

Methods

Patients

This study enrolled consecutive patients who underwent coronary angiography from November 2012 to November 2013 at the Department of Cardiology, National Defense Medical College (Tokorozawa, Japan). Patients undergoing hemodialysis, those receiving treatment for cancer, and those with infections, autoimmune disease, vasculitis, myocarditis, cardiomyopathy, congenital heart disease, pulmonary arterial hypertension, cardiac amyloidosis, or severe valvular heart disease were excluded from the study.

This study was approved by the Ethics Committee of National Defense Medical College (No. 1084) and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Coronary angiography and measurement of amino acids

Coronary angiography was performed using the 4Fr catheter system, and angiograms were obtained from 4 standard projections for each right and left coronary artery. Obstructive stenosis was defined as > 75% visual lumen narrowing. CAD was defined as the presence of coronary stenosis in at least 1 major coronary artery or its branches, or any clinical history of myocardial infarction, percutaneous coronary intervention (PCI), or coronary artery bypass surgery (CABG). Blood samples were collected through a guiding sheath during coronary angiography before intravenous heparin administration and immediately stored on ice. Serum was obtained by centrifugation at 3,000 rpm for 10 min at 4℃. Serum levels of hydroperoxides were measured as an index of oxidative stress using the reactive oxygen metabolites (d-ROMs) test[19]. Other serum aliquots were stored at -80℃ until analysis. Serum levels of L-arginine, L-citrulline, L-ornithine, ADMA, and symmetric dimethylarginine (SDMA) were measured by high-performance liquid chromatography using a Shimadzu RF-20A system (Shimadzu Corp., Kyoto, Japan) with a Symmetry C18 column (3.9 × 150 mm, 5 μm particle size; Waters Corp., Milford, MA). The detection method was based on fluorescent derivatisation with AccQ-Fluor™ reagent (Waters Corp.) according to previously described methods[20,21].

Patient classification

Patients were divided into three groups: the CAD (–) group included stable patients without coronary stenosis or history of coronary intervention; the CAD (+) group included stable patients with CAD; and the ACS group included patients with unstable angina pectoris (UAP) or with acute myocardial infarction (AMI). We diagnosed ACS according to American College of Cardiology/American Heart Association guidelines[22].

Coronary risk factors

Coronary risk factors were assessed using the following definitions. Hypertension was defined as blood pressure over 140/90 mmHg or prior diagnosis of hypertension with blood pressure-lowering medication. Diabetes mellitus was defined as fasting blood glucose > 126 mg/dL or use of insulin or oral hypoglycemic agents. Hyperlipidemia was defined as total cholesterol > 220 mg/dL, low-density lipoprotein (LDL) cholesterol > 140 mg/dL or prior diagnosis of hyperlipidemia with lipid-lowering medication. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease equation modified for the Japanese population[23,24].

Statistical analysis

Summary data are presented as the mean ± standard deviation with 95% confidence interval for parametric variables or the median (interquartile range) for non-parametric variables. The Kolmogorov-Smirnov test was used to identify distribution patterns. Cross-table analyses were performed using chi-squared test or Fisher's exact test when appropriate. Analysis of variance with the Bonferroni post-hoc test was used for comparisons among more than three

Table 1. Baseline characteristics

	CAD (–) (n=38)	CAD (+) (n=56)	ACS (n=40)	p-value
Age (years)	66 ±13	69 ±8	68 ± 8	0.253
Sex (women/men), n (%)	8/30 (21/79)	9/47 (16/84)	4/36 (10/90)	0.404
BMI (kg/m^2)	24 ± 4	23 ± 4	24 ± 3	0.625
Hypertension, n (%)	24 (63)	42 (75)	27 (68)	0.451
Hyperlipidemia, n (%)	19 (50)	39 (70)	25 (63)	0.156
Diabetes mellitus, n (%)	13 (34)	22 (39)	17 (43)	0.751
Current smoking, n (%)	13 (34)	16 (29)	16 (40)	0.503
Previous condition				
OMI, n (%)	0	16 (29)	10 (25)	**0.002**
CABG, n (%)	0	4 (7)	1 (3)	0.178
PCI, n (%)	0	27 (48)	10 (25)	**<0.001**
Atrial fibrillation, n (%)	7 (18)	0	0	**0.001**
Coronary angiography and treatment				
Syntax score	0 (0, 6)	15 (6, 23)	18 (11, 26)	**<0.001**
AMI, n (%)	0	0	13 (33)	**<0.001**
Revascularization, emergent, n (%)	0	0	20 (50)	**<0.001**
Revascularization, total, n (%)	0	14 (25)	38 (95)	**<0.001**
Medication				
Beta-blocker, n (%)	7 (18)	22 (39)	17 (43)	**0.048**
ACE inhibitor, n (%)	4 (11)	4 (7)	2 (5)	0.645
ARB, n (%)	11 (29)	34 (61)	16 (40)	**0.007**
Carcium channel blocker, n (%)	16 (42)	28 (50)	11 (28)	0.086
Furosemide, n (%)	5 (13)	5 (9)	2 (5)	0.451
Spironolactone, n (%)	1 (3)	2 (4)	1 (3)	0.944
Statins, n (%)	10 (26)	43 (77)	21 (53)	**<0.001**
Insulin, n (%)	3 (8)	4 (7)	2 (5)	0.866
Aspirin, n (%)	13 (34)	45 (80)	22 (55)	**<0.001**

Emergent revascularization indicates percutaneous or surgical coronary intervention subsequently performed after emergent coronary angiography. Total revascularization includes emergent and ad-hoc coronary interventions performed before discharge.
CAD: coronary artery disease, ACS: acute coronary syndrome, BMI: body mass index, OMI: old myocardial infarction, CABG: coronary artery bypass surgery, PCI: percutaneous coronary intervention, AMI: acute myocardial infarction, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker.
Significant p-values are shown in bold.

groups. Kruskal-Wallis test was employed for non-parametrical variables. Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL). A 2-sided p-value of less than 0.05 was considered statistically significant.

Results

Study population

A total of 134 patients including 40 with ACS were enrolled (**Table 1**). Among the 94 stable patients who underwent elective coronary angiography, 38 and 56 patients were included in the CAD (–) and CAD (+) groups, respectively. Atrial fibrillation was observed only in patients in the CAD (–) group. The CAD (+) group included 11 patients in whom no significant coronary stenosis remained at angiography. Fourteen patients in the CAD (+) group required coronary intervention; 12 and 2 patients underwent PCI and CABG, respectively. The ACS group comprised 13 patients with AMI and 27 patients with UAP. Among the 40 ACS patients, 20 underwent emergent revascularization on the same day as coronary angiography (PCI 18, CABG 2), another 18 patients underwent coronary intervention during the same hospital admission (PCI 13, CABG 5), and the remaining 2 patients were treated with medication alone because their coronary stenosis was not suitable for intervention.

There were no differences in age or medical history of hypertension, hyperlipidemia, or diabetes mellitus among the groups. However, angiotensin receptor blockers, statins, and aspirin were used less frequently in the CAD (–) group. Laboratory examination revealed that the high-density lipoprotein cholesterol level was higher in CAD (–) patients compared with the other groups, whereas LDL cholesterol level was not different among the three groups (**Table 2**). Other measurements of coronary risk factors such as hemoglobin A_{1C}, eGFR, and brain natriuretic peptide did not show any significant differences among the groups.

Comparison of L-arginine and its related metabolites

Box plots of L-arginine metabolites are shown in **Figure 1**. There were no significant differences in serum L-arginine and L-ornithine levels between the groups (**Table 2**, **Figure**

Table 2. Amino acids and biomarkers

	CAD (–) (n=38)	CAD (+) (n=56)	ACS (n=40)	p-value
L-arginine (μmol/L)	88 ± 21	91 ± 21	86 ± 26	0.514
L-ornithine (μmol/L)	58 ± 31	63 ± 31	63 ± 31	0.657
L-citrulline (μmol/L)	135 ± 62	148 ± 68	174 ± 79*	**0.043**
L-homocysteine (μmol/L)	6.7 ± 4.6	6.1 ± 2.8	5.9 ± 2.2	0.976
L-cysteine (μmol/L)	179 ± 48	190 ± 57	173 ± 49	0.279
ADMA (μmol/L)	0.52 ± 0.15	0.57 ± 0.21	0.54 ± 0.20	0.435
SDMA (μmol/L)	0.73 ± 0.30	0.72 ± 0.34	0.72 ± 0.35	0.989
ADMA/SDMA	0.80 ± 0.30	0.86 ± 0.26	0.84 ± 0.33	0.639
L-arginine/ADMA	178 ± 67	173 ± 76	165 ± 76	0.714
L-arginine/L-citrulline	0.74 ± 0.28	0.70 ± 0.25	0.55 ± 0.19**	**0.001**
L-arginine/L-ornithine	1.94 ± 1.02	1.75 ± 0.81	1.68 ± 0.89	0.408
GABR	0.51 ± 0.19	0.49 ± 0.17	0.39 ± 0.12**	**0.003**
D-ROMs (U.CARR.)	356 ± 74	324 ± 80	323 ± 93	0.129
WBC (/μL)	5626 ± 1435	6144 ± 1499	7517 ± 2540**	**<0.001**
Hb (mg/dL)	13.5 ± 1.7	13.4 ± 1.6	13.8 ± 1.9	0.590
LDL cholesterol (mg/dL)	109 ± 30	99 ± 26	103 ± 31	0.231
TG (mg/dL)	118 (80, 181)	114 (88, 175)	98 (81, 168)	0.760
HDL cholesterol (mg/dL)	60 ± 21#	50 ± 12	49 ± 13	**0.001**
HbA_{1C} (mg/dL)	6.1 ± 1.6	6.0± 1.1	6.0 ± 0.8	0.722
Uric acid (mg/dL)	5.7 ± 1.6	6.1 ± 1.7	6.0 ± 1.5	0.381
CPK (mg/dL)	85 (67, 124)	101 (58, 152)	118 (69, 197)	0.254
eGFR (mL/min)	71 ± 23	62 ± 18	65 ± 17	0.074
CRP (mg/dL)	0.3 (0.3, 0.6)	0.3 (0.3, 0.3)	0.3 (0.3, 0.4)	0.702
$Log_{10}BNP$	1.58 ± 0.67	1.59 ± 0.56	1.67 ± 0.60	0.777

CAD: coronary artery disease, ACS: acute coronary syndrome, ADMA: asymmetric dimethylarginine, SDMA: symmetric dimethylarginine, D-ROMs: derivatives of the reactive oxidative metabolites, LDL: low-density lipoprotein, TG: triglyceride, HDL: high-density lipoprotein, HbA_{1C}: hemoglobin A_{1C}, CPK: creatine phosphokinase, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, BNP: brain natriuretic peptide, $Log_{10}BNP$: log-transformed brain natriuretic peptide (pg/ml).

Significant p-values are shown in bold.

*: p<0.05 vs CAD (–), **: p<0.05 vs CAD (–) and CAD (+), #: p<0.05 vs CAD (+) and ACS in Bonferroni's post-hoc test.

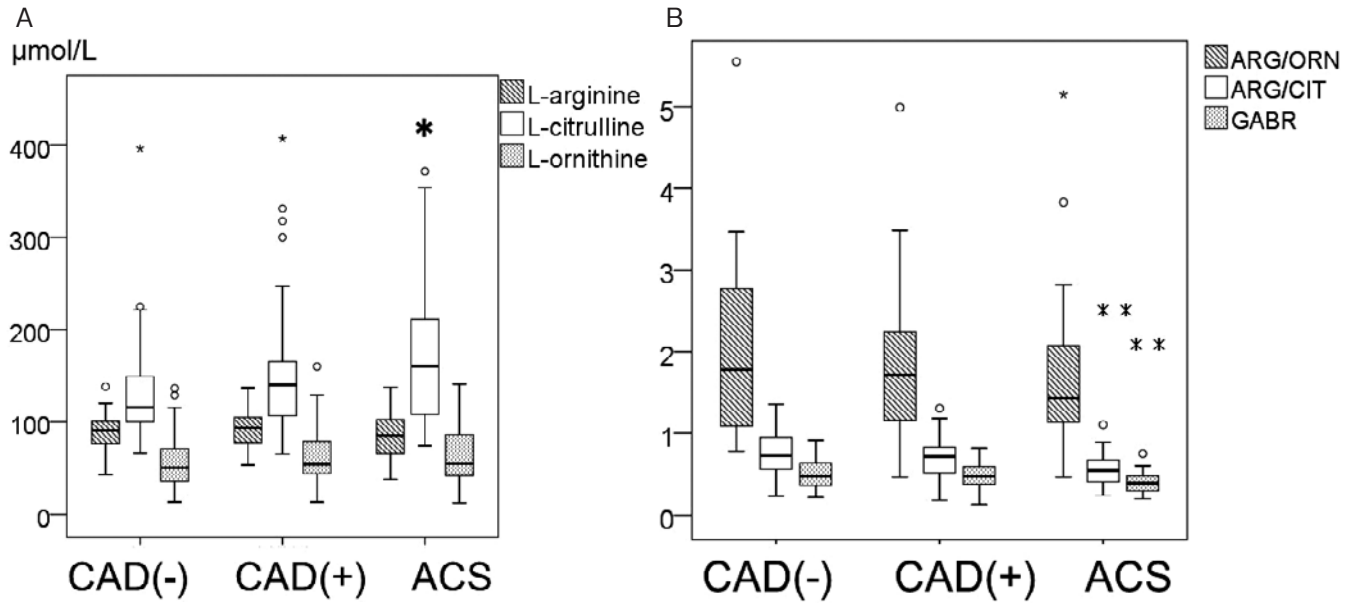

Figure 1. L-arginine and its metabolites

A: Serum concentrations of L-arginine, L-citrulline, and L-ornithine, B: GABR and L-arginine to L-citrulline ratio.

CAD: coronary artery disease, ACS: acute coronary syndrome, ARG: L-arginine, CIT: L-citrulline, ORN: L-ornithine, GABR: global L-arginine bioavailability ratio (defined as L-arginine/[L-ornithine+L-citrulline]).

*: p<0.05 vs CAD (–), **: p<0.05 vs CAD (–) and CAD (+).

1A), whereas serum L-citrulline levels were increased in the ACS group. The levels of ADMA, SDMA, and d-ROMs were not significantly different among the groups (**Table 2**). The ratio of L-arginine to L-citrulline and GABR were lowest in the ACS group (**Figure 1B**). In the ACS group, there were no differences between AMI (n = 13) and UAP (n = 27) in GABR (AMI: 0.38 ± 0.12, UAP: 0.40 ± 0.13; p = 0.724), L-arginine to L-citrulline ratio (AMI: 0.51 ± 0.19, UAP: 0.57 ± 0.19; p = 0.348), or L-arginine to L-ornithine ratio (AMI: 1.94 ± 1.09, UAP: 1.55 ± 0.76; p = 0.196).

Discussion

The major findings of this study are as follows. In patients with ACS, GABR was significantly decreased compared with stable patients with or without CAD, suggesting the presence of decreased arginine bioavailability in patients with ACS. Reduced GABR was due to the elevated serum level of L-citrulline with no change in serum level of L-arginine, which could be explained by relative L-arginine deficiency due to increased NO production. The serum levels of L-ornithine and ADMA and the L-arginine to L-ornithine ratio were not significantly different among the three groups.

Although the source of increased serum L-citrulline in patients with ACS observed in this study is unknown, one possibility is that it could in part be caused by increased production of NO by inducible NOS (iNOS) due to systemic inflammation in ACS. ACS is known to be accompanied by a systemic inflammatory response, as indicated by increased circulating levels of inflammatory markers such as high-sensitivity C-reactive protein (CRP), interleukin-6, and tumor necrosis factor α[25-27]. Systemic inflammation induces iNOS expression in various types of cells and augments NO production. Sánchez de Miguel et al reported higher protein expression levels of iNOS and increased NO production in neutrophils from patients with ACS compared with those from healthy donors[28]. They measured accumulation of tritium-labeled citrulline in tritium-labeled arginine-loaded neutrophils as a marker of NO production and showed that the amount of tritium-labeled citrulline accumulated in neutrophils from patients with ACS was significantly higher than in neutrophils from healthy donors. In our study, the increased white blood cell counts in patients with ACS suggested the presence of systemic inflammation, so the possibility that increased serum levels of L-citrulline could be derived from increased production of L-citrulline by iNOS induced by systemic inflammation in the setting of ACS cannot be excluded.

Among the stable patients who underwent coronary angiography in the present study, GABR was not different between those with and without CAD, which may be due to similar prevalence of coronary risk factors such as diabetes mellitus. There have been two reports regarding GABR in patients with stable CAD[15,16]. Tang et al measured plasma levels of L-arginine and its related metabolites in 1010 consecutive patients who underwent coronary angiography[15]. They found lower plasma L-arginine, higher plasma L-ornithine and L-citrulline, and lower GABR in patients with compared to those without obstructive CAD. After adjusting for Framingham risk score, CRP, and creatinine clearance, high levels of L-ornithine and L-citrulline and low GABR, but not low L-arginine levels, remained significantly associated with obstructive CAD. Only high L-citrulline level and low GABR were associated with subsequent risk of major adverse cardiovascular events (MACE) over 3 years. Sourij et al measured GABR in 2236 patients who underwent coronary angiography and reported that decreased GABR was associated with increased cardiovascular mortality during 7.7 years of follow-up[16], which was consistent with the findings of Tang et al[15]. As for the association between GABR and prevalence of obstructive CAD, however, the results of Sourij et al[16] were not consistent with those of Tang et al. In their analysis, although GABR was associated with the prevalence of obstructive CAD in the crude logistic model, this association was no longer significant after adjustment for the presence of diabetes mellitus. In the present study, the prevalence of diabetes mellitus was not significantly different between patients with and without obstructive CAD, and GABR was not associated with the presence of obstructive CAD, which is consistent with the results of Sourij et al[16].

ADMA is an endogenous inhibitor of NOS, and its serum level is reported to be increased in patients with stable CAD or with ACS[29], which is also associated with subsequent incidence of cardiovascular events[30]. In this study, however, serum levels of ADMA were not different between the three groups. Increased serum ADMA level causes endothelial dysfunction and was reported to be associated with increased risk of cardiovascular events in a broad range of patients, including stable patients with or without CAD and in patients with ACS, as well as in healthy individuals[30]. However, whether increased serum ADMA level is an independent predictor of the presence of CAD remains controversial. Wang et al measured plasma ADMA levels in 1011 consecutive patients who underwent elective coronary angiography, and reported that after adjusting for Framingham risk score, CRP, and creatinine clearance, plasma ADMA level was no longer associated with prevalence of obstructive CAD, although it remained an independent predictor of incident MACE[31]. Xuan et al performed a meta-analysis of 16 case-control studies, which included 4713 participants in 20 cohorts. They found that 12 cohorts demonstrated a positive association and the remaining 8 cohorts a negative association between ADMA and the risk of CAD, and overall, it was concluded that ADMA level is significantly increased in patients with CAD compared with healthy controls[29]. However, Wang et al noted that they could not adjust for several confounding factors such as smoking, age, and sex, which could have overestimated the association between ADMA and CAD. The serum ADMA level is reported to be increased by oxidative stress in the presence of coronary risk

factors such as hypertension, hyperlipidemia, diabetes mellitus, and smoking. In the present study, the prevalence of these risk factors was not significantly different among the three groups. The levels of oxidative stress measured by d-ROMs test were also not different, which was consistent with unchanged ADMA levels among the three groups. In patients with coronary risk factors, endothelial dysfunction of the coronary artery indicated by vasoconstrictive response to intracoronary acetylcholine infusion preceded the development of atherosclerosis detectable by intracoronary ultrasound imaging, suggesting that endothelial dysfunction may have a role in the initiation of atherosclerosis[32]. Because decreased GABR or elevated serum ADMA levels are causes of endothelial dysfunction, they may be useful predictors of initiation and progression of atherosclerosis rather than markers of the presence of atherosclerosis.

Study limitations and clinical implications

In this study, blood samples were collected through a guiding sheath during coronary angiography, so in cases of acute coronary syndrome blood samples were obtained in the emergency setting and patients were not always fasted, which means that measured values were influenced by a meal. Plasma arginine is reported to exhibit a postprandial increase with a peak concentration approximately 3 h after the meal[33]. This effect of a meal could cause overestimation and blunt the decrease in arginine level in patients with acute coronary syndrome. However, the increased L-citrulline levels in patients with acute coronary syndrome was not explained by the effect of a meal alone. Pharmacokinetic analysis showed that oral ingestion of L-citrulline increased its serum level with a peak within 1 h after ingestion. It has been reported that after entering the circulation, L-citrulline is rapidly converted to L-arginine and L-ornithine within 1 h, which increased both L-arginine and L-ornithine levels in proportion to the increase in L-citrulline levels[34]. In the present study, serum L-citrulline level increased without a significant increase in either L-arginine or L-ornithine levels, resulting in decreased GABR in patients with acute coronary syndrome. The decreased GABR was attributed to decreased arginine bioavailability rather than the effect of a meal, because neither L-arginine nor L-ornithine levels were changed. To confirm this, however, further study is needed to clarify the effect of a meal on GABR.

In addition to the effect of a meal, circadian variation should be considered. The existence of cardiovascular circadian rhythms is well known. Endothelial function measured by endothelium-dependent flow-mediated vasodilation in brachial arteries (FMD) has been reported to show diurnal variation with a significant attenuation in the morning[35]. It is speculated that serum concentration of arginine and its elated metabolites may also show diurnal variation. Plasma arginine levels were reported to show circadian variation with an arginine-free diet. Plasma L-arginine concentration was relatively high in the morning, decreased during the awake period, and returned to its baseline level overnight[33]. To our knowledge, there have been no reports about circadian variation of GABR. Only one paper suggested that serum ADMA concentration has diurnal variation with a morning peak[36], which is the opposite pattern to that of endothelial function. In the present study, time of day of blood sampling varied depending on the schedule of coronary angiography, which could obscure the differences in GABR and ADMA values between patients with or without CAD because of diurnal variation. To use GABR or ADMA as markers of coronary risk, circadian variation of these values must be clarified.

In this study, levels of arginine and its metabolites were measured at the whole-body level and their intracellular levels were unknown. Thus, it is not clear whether the increased serum L-citrulline level observed in patients with ACS was derived from increased production of L-citrulline within NO-producing cells, such as endothelial cells, macrophages, and neutrophils. Although production of NO and L-citrulline are reported to be increased in neutrophils from patients with ACS, it is also unclear whether increased intracellular L-citrulline causes elevation of circulating L-citrulline, because intracellular citrulline is recycled to arginine for NO synthesis within the cell. Further study is needed to identify the source of increased serum citrulline observed in this study in patients with ACS.

To predict arginine bioavailability, which is one of the major determinants of endothelial function, we measured GABR but did not measure endothelial function as FMD or vasomotor responses to acetylcholine in coronary arteries. Therefore, the association between GABR and endothelial function has not been validated in this study. If decreased GABR is confirmed to be an index of endothelial dysfunction in ACS, those patients who will benefit from arginine supplementation could be identified. Although a previous study demonstrated that arginine supplementation in patients with acute myocardial infarction was not beneficial[37], supplementation may be beneficial for such patients with low GABR.

Conclusion

In conclusion, increased serum levels of L-citrulline and decreased GABR were observed in patients with ACS, suggesting the presence of relative arginine deficiency in ACS.

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Conflicts of Interest
None.

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