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Acute Effects of Vasoactive Drug Treatment on Brachial Artery Reactivity

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OBJECTIVES The goal of this study was to investigate whether concomitant therapy with vasoactive medications alters the results of noninvasive assessment of endothelial function.

BACKGROUND Ultrasound assessment of brachial artery flow-mediated dilation is emerging as a useful clinical tool. The current practice of withholding cardiac medications before ultrasound studies has unknown utility and would limit the clinical use of the methodology.

METHODS To determine whether a single dose of a vasoactive drug influences brachial reactivity, we examined flow-mediated dilation and nitroglycerin-mediated dilation in 73 healthy subjects (age 27 ± 6 years). Studies were completed at baseline and 3 h after randomized treatment with a single oral dose of placebo, felodipine (5 mg), metoprolol (50 mg), or enalapril (10 mg). To determine if holding vasoactive therapy for 24 h before study yields different results than continuation of clinically prescribed medications, we examined vascular function in 72 patients (age 57 ± 10 years) with coronary artery disease. Ultrasound studies were performed 24 h after the last dose and again 3 h after patients took their clinically prescribed medications.

RESULTS In healthy subjects one dose of all three drugs lowered blood pressure, and metoprolol also lowered heart rate. However, there was no significant effect of treatment on brachial artery dilation. In patients with coronary artery disease on chronic treatment, taking prescribed medications reduced blood pressure and heart rate, but had no significant effect on brachial artery dilation.

CONCLUSIONS Recent administration of commonly used nonnitrate vasoactive drugs has no significant effect on brachial reactivity. These findings suggest that current practice of withholding cardiac medications before testing endothelial function may not be necessary, making this methodology more practical for clinical use. (J Am Coll Cardiol 2002;40:761–5) © 2002 by the American College of Cardiology Foundation

The vascular endothelium plays a critical role in the regulation of vascular tone, inflammation, and thrombosis. Impaired endothelial function in individuals with atherosclerosis and coronary risk factors contributes to the pathophysiology of acute cardiovascular syndromes (1). Ultrasound examination of brachial artery flow-mediated dilation (FMD) has emerged as a valuable noninvasive method for assessing endothelial function and is developing into a potentially useful tool in the clinical setting (2). Recent studies in the coronary and forearm circulation demonstrate that endothelial dysfunction is an independent predictor of future cardiovascular events (3–7), highlighting the prognostic importance of identifying abnormalities in vascular function in individual patients. Noninvasive assessment of endothelial function may prove valuable in cardiovascular risk assessment and become a target for future cardiovascular risk reduction.

Although brachial artery reactivity appears promising, its broad use has been limited by lack of standardized methodology. The technique is highly operator dependent and may vary according to technical factors such as arm cuff position, image quality, method of analysis, and timing of studies (8). One pressing methodological issue limiting the clinical and research utility of this technique is the current practice of withholding cardiac medications for at least 24 h before study. Although the premise for this practice (removing confounding effects of vasoactive medications) makes intuitive sense, there is little data to support it. Thus, the purpose of this study was to investigate whether acute oral administration of nonnitrate vasoactive agents commonly used in clinical practice would have any influence on brachial artery reactivity.

METHODS

Research subjects. We studied two groups of subjects. One group consisted of normal healthy volunteers recruited by advertisement who were excluded if they had a clinical history of hyperlipidemia, diabetes mellitus, hypertension, chest pain, claudication, prior stroke, or family history of premature coronary artery disease (CAD). Subjects were also excluded if they were pregnant, used tobacco, or were taking any medications including antioxidant vitamins and estrogen/progesterone supplements.

The second group included patients with angiographi-

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ANOVA = analysis of variance
BMI = body mass index
CAD = coronary artery disease
FMD = flow-mediated dilation
NMD = nitroglycerin-mediated dilation

cally documented CAD (at least one coronary stenosis >50%). Exclusion criteria included unstable angina, uncontrolled hypertension, clinically significant valvular heart disease, congestive heart failure, or any other condition that would preclude safely withholding vasoactive medications as required for the protocol. Written informed consent was obtained, and the Institutional Review Board of Boston Medical Center approved the protocol.

Study protocols. NORMAL SUBJECTS. After fasting overnight, each subject rested quietly in a supine position for 15 min, and baseline vital signs were recorded. Blood pressure was measured using an automated monitor (Dinamap XL, Johnson and Johnson Medical, Arlington, Texas). Subjects underwent baseline examination of endothelium-dependent and -independent vasodilation of the brachial artery, as previously described (9,10). Briefly, two-dimensional and pulsed Doppler flow velocity signals were obtained from the brachial artery at baseline and during reactive hyperemia induced by 5-min arterial occlusion with a cuff on the upper arm. The same arterial segment was imaged on all subsequent studies based on anatomical landmarks. After a 10-min rest period to allow restoration of baseline conditions, brachial artery dilation was assessed before and 3 min after administration of sublingual nitroglycerin (0.4 mg). If the subject had a history of migraine headaches, systolic blood pressure <100 mm Hg, or adverse reaction to nitroglycerin, this portion of the protocol was omitted. Brachial artery images were acquired using Toshiba 140 SSHA or Toshiba Powervision 6000 ultrasound systems equipped with a 7.5-MHz transducer. Brachial images were digitized at end diastole using an R-wave trigger. Personnel blinded to both image sequence and treatment assignment measured brachial arterial diameter and flow-velocity integral using customized software (9).

In a double-blind fashion, participants were consecutively randomized to treatment with a single oral dose of placebo, felodipine (5 mg), metoprolol (50 mg), or enalapril (10 mg). Three hours after treatment, vital signs were recorded, and brachial artery ultrasounds were repeated. A 3-h time point was chosen because it represents a period within the window for an acute physiologic drug effect based on known pharmacokinetics of these medications.

SUBJECTS WITH CAD. Patients with CAD were asked to withhold all vasoactive medications for 24 h, not smoke for at least 24 h and to fast overnight before evaluation. After a 15-min supine rest period, baseline vital signs were recorded and brachial artery studies performed as described above. Subjects then took their usual morning antianginal and/or antihypertensive medications as prescribed by their physicians, except for oral or topical nitrates. Three hours after treatment, vital signs were recorded and brachial artery studies were repeated. Patient medications were recorded and classified as beta-blocker, calcium channel blocker, aspirin, or angiotensin-converting enzyme (ACE) inhibitor/angiotensin receptor blocker.

Statistical analyses. In normal subjects the effects of treatment on vascular function and hemodynamics were examined using two-way repeated measures analysis of variance (ANOVA) with Student-Newman-Keuls post hoc comparison. The two factors in this analysis were time (before and after treatment) and drug (placebo, felodipine, metoprolol, and enalapril) with brachial artery diameter, FMD, nitroglycerin-mediated dilation (NMD), systolic and diastolic blood pressure, and heart rate as dependent variables. In patients with CAD, the effect of treatment on brachial artery responses, vessel size, and hemodynamic parameters were examined using the paired Student *t* tests.

To compare the effect of vasoactive medication administration on the reproducibility of FMD in the normal subjects, the absolute value of the difference between the baseline and follow-up studies was calculated for each treatment (placebo, felodipine, metoprolol, and enalapril) and compared using one-way ANOVA. For subjects with coronary disease, the absolute value of the difference between baseline and follow-up study was calculated and compared with that observed in a prior study from our laboratory (11) using the unpaired *t* test. All data are presented as mean ± SD unless otherwise indicated. A p value <0.05 was considered significant. Statistical analysis was performed with Sigma Stat for Windows 2.03 software (SPSS Inc., Chicago, Illinois).

RESULTS

Clinical characteristics. A total of 145 subjects were studied. The group of normal healthy subjects (n = 73) had mean age 27 ± 6 years, body mass index (BMI) 24 ± 3 kg/m^2 and were 66% male. The group of patients with CAD (n = 72) had mean age 57 ± 10 years, BMI 30 ± 7 kg/m^2, and were 78% men. Of the patients with CAD, 64% had a history of hypertension, 79% had hypercholesterolemia, 47% had a history of smoking, and 25% had a clinical history of diabetes mellitus. The number and types of medications taken by the patients with coronary disease are displayed in Table 1.

Effects of single-dose treatment in healthy subjects. For the entire group of normal subjects, mean baseline brachial artery FMD was 9.5 ± 5.3% and NMD 17.8 ± 6.6%, brachial artery diameter 3.8 ± 0.7 mm, resting systolic blood pressure 123 ± 11 mm Hg, and heart rate 69 ± 10 beats/min. Hemodynamics and brachial artery parameters before and 3 h after treatment are shown in Table 2, and the

Table 1. Prescribed Medications in the Patients With Coronary Artery Disease

Medication Type	Number of Patients (%) Total = 72
ACE inhibitor	29 (40)
Angiotensin receptor antagonist	4 (6)
Beta-blocker	60 (83)
Calcium channel blocker	21 (29)
Aspirin	66 (92)
Taking medications from one class	3 (4)
Taking medications from two classes	34 (47)
Taking medications from three or more classes	35 (49)

ACE = angiotensin-converting enzyme.

changes in these parameters are displayed in Figure 1. All three classes of drugs significantly lowered systolic and mean arterial pressure 3 h after treatment, and subjects who received metoprolol experienced a significant reduction in heart rate. Placebo had no significant effect on hemodynamic or brachial artery parameters. The study had 80% power (alpha = 0.05) to detect a change of 2.4 percentage points in FMD in any one group.

Effect of morning dose of vasoactive medication on hemodynamics and vascular function. In contrast to normal subjects, patients with CAD had impaired brachial artery dilator responses, consistent with previous studies (10). For the entire group, baseline FMD was 7.5 ± 4.4% ($p = 0.016$ compared with normal subjects), and NMD was 13.3 ± 6.3% ($p = 0.002$ compared with normal individuals). As shown in Table 3, blood pressure and heart rate were significantly lower than baseline 3 h after the subjects took their morning medications. In contrast, baseline diameter, FMD and NMD were unaffected. The study had 80% power (alpha = 0.05) to detect a change of 1.2 percentage points before and after medication treatment.

As shown in Table 1, 40% of patients were taking an ACE inhibitor as part of their prescribed therapy. As for the group as a whole, vascular function in this subgroup was unaffected by treatment. Brachial artery FMD was 7.0 ± 4.3% at baseline and 6.9 ± 4.5% 3 h later ($p = 0.91$). In individuals treated with the tissue-specific ACE inhibitor quinapril ($n = 6$), FMD was 6.9 ± 6.5% at baseline and 6.4 ± 4.7% 3 h after the dose ($p = 0.64$).

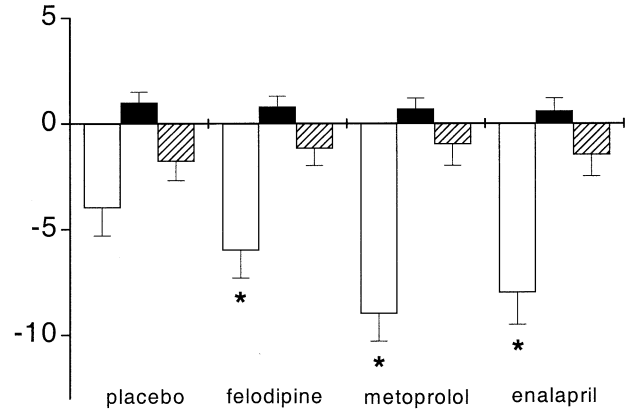

Figure 1. The effect of a single oral dose of vasoactive medication on systolic blood pressure (SBP) (mm Hg) and absolute percent change in brachial artery flow-mediated (FMD) and nitroglycerin-mediated dilation (NMD) in normal subjects. Examinations were performed at baseline and 3 h after treatment with either placebo, felodipine (5 mg), metoprolol (50 mg), or enalapril (10 mg). *All three drugs lowered blood pressure significantly ($p < 0.05$) but had no effect on FMD or NMD. **Open bar** = change in SBP (mm Hg); **solid bar** = change in FMD (%); **hatched bar** = change in NMD (%).

Effect of prescribed medications on reproducibility. To investigate whether taking medication altered the reproducibility of FMD when measured twice over a 3-h period, we compared the reproducibility of FMD in each group of normal patients. The absolute value of the differences between baseline and follow-up study were 2.9 ± 1.7, 2.4 ± 1.6, 2.4 ± 2.3, and 2.7 ± 2.6 percentage points, for the placebo, felodipine, metoprolol, and enalapril groups, respectively ($p = 0.84$ by one-way ANOVA). For the patients with coronary disease, we examined the reproducibility of the baseline and follow-up study (with routine medications administered between studies). This reproducibility was compared with the reproducibility observed in a prior study from our laboratory that involved a separate cohort of 43 patients with CAD and study of FMD before and 2 h after administration of placebo (11). The absolute difference between baseline and follow-up study was 2.5 ± 2.5 percentage points in the present study and 2.1 ± 2.8 percentage points in the prior study with placebo given between studies ($p = 0.42$). Thus, when vasoactive medications are administered between studies, reproducibility is

Table 2. Hemodynamic and Brachial Parameters Before and After Drug Treatment in Healthy Subjects

	Placebo (n = 20)		Felodipine (n = 19)		Metoprolol (n = 20)		Enalapril (n = 14)	
	Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2
SBP (mm Hg)	121 ± 6	117 ± 6	125 ± 6	119 ± 6*	123 ± 6	113 ± 6*	124 ± 6	116 ± 6*
DBP (mm Hg)	67 ± 5	66 ± 5	69 ± 6	65 ± 6*	69 ± 6	67 ± 6	71 ± 5	65 ± 5*
MAP (mm Hg)	85 ± 5	83 ± 5	88 ± 5	83 ± 5*	87 ± 5	82 ± 5*	89 ± 4	82 ± 4*
HR (beats/min)	67 ± 7	67 ± 7	68 ± 7	71 ± 7	74 ± 7	65 ± 7*	67 ± 7	69 ± 7
FMD (%)	10.6 ± 2.2	11.6 ± 2.2	8.6 ± 2.2	9.4 ± 2.2	8.3 ± 2.2	9.0 ± 2.2	10.9 ± 2.2	11.5 ± 2.2
NMD (%)	18.9 ± 2.8	17.1 ± 2.8	19.5 ± 2.6	18.3 ± 2.6	15.0 ± 3.6	14 ± 3.6	16.5 ± 3.1	15 ± 3.1
Brachial diameter (mm)	3.53 ± 0.11	3.52 ± 0.11	3.78 ± 0.11	3.83 ± 0.11	3.97 ± 0.11	3.99 ± 0.11	3.93 ± 0.10	3.86 ± 0.10
Reactive hyperemia (%)	501 ± 276	634 ± 276	751 ± 278	552 ± 278	723 ± 278	664 ± 278	823 ± 279	730 ± 279

*$p < 0.05$ by repeated measures analysis of variance (baseline vs. 3 h, within each treatment group). Data are mean ± SD.

DBP = diastolic blood pressure; FMD = flow-mediated dilation; HR = heart rate; MAP = mean arterial pressure; NMD = nitroglycerin-mediated dilation; SBP = systolic blood pressure; Visit 1 = baseline; Visit 2 = 3 h after treatment.

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Table 3. Brachial Responses and Hemodynamics After Treatment in Patients With Coronary Artery Disease

	Baseline	After Treatment	p Value
SBP (mm Hg)	134 ± 18	128 ± 20	<0.001
DBP (mm Hg)	74 ± 10	72 ± 11	0.008
MAP (mm Hg)	94 ± 12	90 ± 13	<0.001
HR (beats/min)	65 ± 12	62 ± 11	0.007
FMD (%)	7.5 ± 4.4	7.6 ± 4.8	0.97
NMD (%)	13.3 ± 6.3	13.4 ± 6.5	0.85
Brachial diameter (mm)	4.6 ± 0.7	4.7 ± 0.8	0.08
Reactive hyperemia (%)	585 ± 379	623 ± 304	0.68

Data are mean ± SD.
Abbreviations as in Table 2.

comparable with that observed when placebo is administered between studies.

DISCUSSION

This study demonstrated that treatment with a single dose of commonly used antihypertensive and antianginal medication lowers blood pressure and heart rate, but has no effect on resting brachial artery size, FMD, NMD, and the reproducibility of FMD. These results were obtained when healthy patients received single doses of specific agents for the first time and when patients on chronic therapy for CAD are studied before and after receiving their clinically prescribed medications. These findings suggest that acute alterations in systemic hemodynamics and/or local resting arterial tone induced by these medications do not alter the capacity of the brachial artery to respond to endothelium-derived and exogenous vasodilators.

No previous study examined the specific question addressed in the current study. In most prior studies of endothelial function in human subjects, all vasoactive medications were withheld for at least 24 h, and a recent paper recommended withholding medications for four half-lives (2) because of the concern that concurrent treatment would confound the results. The present study raises the possibility that this practice may not be necessary in some circumstances. While prescribed medications have been safely withheld for 48 h in studies involving relatively large numbers of patients with CAD (12) and for up to 14 days in patients with hypertension (13), it certainly has a theoretical risk in patients with cardiovascular disease. When it is not possible to closely monitor patients or make clinical stability a criterion for eligibility (as was done in the present study), the practice of withholding medications may be less appropriate. For example, an ongoing study of endothelial function in the Framingham Heart Study cohort does not require that medications be withheld, although the large sample size in that study will permit statistical adjustment for medications (14). In spite of the present findings, it remains likely that the study design of smaller scale and intervention studies will continue to require that vasoactive medications be withheld because of the possibility of interactions between prescribed medications and the intervention of interest.

The requirement to withhold vasoactive medications will also be an important issue if assessment of endothelial function proves to have clinical utility. There currently is great interest in the possibility that the methodology will be used to assess cardiovascular disease risk and to guide therapy in individual patients (2). This interest is based on the growing evidence that brachial endothelial function provides independent prognostic information with regard to future cardiovascular risk (4–7) and observations that many interventions known to reduce cardiovascular risk also improve endothelial function (15). If patients can undergo testing of endothelial function without regard to their last dose of vasoactive medication, the methodology will be applicable to larger numbers of patients and clinical settings.

Several prior studies have demonstrated beneficial effects of ACE inhibitors on endothelial function, and the negative findings of the present study do not contradict that prior work. For example, enalaprilat infusion acutely improves the endothelium-dependent vasodilator response to acetylcholine in microvessels of the forearm (16) and the leg (17). Several studies have shown improved FMD after more chronic treatment with ACE inhibitors (18,19). It seems likely that the failure to observe an effect of ACE inhibitors in chronically treated patients in the present study indicates that 24 h is an insufficient period of time for the beneficial effects to subside. In support of this possibility is the observation by Anderson et al. (18) that quinapril treatment for eight weeks was associated with improved FMD compared with baseline, even though the follow-up studies were completed 72 h after the last dose of medication. Thus, a longer washout period should be considered if a study requires examination of endothelial function free from the effects of ACE inhibitors.

Study limitations. There are several limitations of this study. In patients with CAD, we did not examine the effects of individual drugs on vascular reactivity but, rather, investigated the effect of all prescribed medications. We cannot exclude the possibility that drug interactions masked an effect of therapy to improve or worsen vasodilator function. This possibility seems less likely given that we observed no effect of the individual drugs in healthy subjects. Examining the effect of all prescribed medications is more applicable to the question of whether the methodology will have clinical utility. The present study examined only a singe agent and dose of the three classes of vasoactive medication. Thus, we cannot exclude the possibility that other agents and/or different doses might have had a different effect on vascular function. Similarly, the effects of longer-term treatment might have had a different effect.

Conclusions. Acute nonnitrate vasoactive drug therapy has no effect on brachial artery vascular responses in healthy subjects or in patients with CAD. These findings suggest that it may not be necessary to hold vasoactive medications

before ultrasound examination, making it more practical for clinical use.

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