Inhibition of angiotensin-converting enzyme and phosphodiesterase type 5 improves endothelial function in heart failure

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ABSTRACT

ACE (angiotensin-converting enzyme) inhibitors and PDE5 (phosphodiesterase type 5) inhibitors have each been reported to improve endothelial function in cardiovascular disease patients, but the comparative and combined effects of these two classes have not been studied previously. We sought to characterize the acute effects of ramipril alone, sildenafil alone, or their combination on endothelial function in patients with CHF (chronic heart failure). CHF subjects (n = 64) were randomized to receive placebo, 10 mg of ramipril alone, 50 mg of sildenafil alone or a combination of ramipril and sildenafil in a double-blind manner. FMD (flow-mediated dilation) of the brachial artery was determined by high-resolution ultrasound imaging before and at 1, 2 and 4 h after administration of the study drug. Ramipril alone increased FMD at 4 h compared with placebo (+2.3 ± 1.3, P = 0.02). Sildenafil alone increased FMD at 1, 2 and 4 h compared with placebo (+3.9 ± 1.4, +4.6 ± 1.8 and +3.7 ± 1.3 % respectively, all P < 0.02). Sildenafil in combination with ramipril increased FMD at 1, 2 and 4 h when compared with placebo (+3.5 ± 1.5, +4.5 ± 1.8 and +4.8 ± 1.3 % respectively, all P < 0.03). Ramipril and sildenafil both acutely improved FMD in patients with CHF, with additive effects evident at 4 h during combination therapy. Therefore further work to characterize chronic effects of combined ACE and PDE5 inhibition on endothelial function are warranted.

INTRODUCTION

Endothelium-dependent NO (nitric oxide)-mediated vasodilation in response to hormonal agonists and shear stress is decreased in the coronary and skeletal muscle circulations of patients with CHF (chronic heart failure) when compared with age-matched normal subjects [1–3]. Decreased endothelium-dependent vasodilation in CHF is attributable to decreased activity of the l-arginine/NO metabolic pathway in endothelial cells, decreased bioavailability of NO due to degradation of NO by reactive oxygen species in the vascular wall and alterations in cGMP signalling in vascular smooth muscle [2,4–6].

Increased NO bioavailability in response to ACE (angiotensin-converting enzyme) inhibition and increased vascular smooth muscle sensitivity to NO in response to PDE5 (phosphodiesterase type 5) inhibition have been reported previously to improve impaired...
endothelium-dependent vasodilation in patients with CHF [4,7–10]. However, the comparative effects of these agents alone and in combination on endothelial function in the intact human circulation have not been characterized previously. Based on findings from experimental studies, we hypothesized that these two classes of drugs may have synergistic actions on NO-mediated endothelium-dependent vasodilation [11–13]. Accordingly, the current randomized placebo-controlled double-blind trial was undertaken to compare prospectively the effects of ACE inhibition with ramipril, PDE5 inhibition with sildenafil, and the combination of these two agents on FMD (flow-mediated vasodilation) in the brachial artery, systemic artery pressure and neurohormonal activation in subjects with CHF.

**METHODS**

**Study population**

Sixty-four subjects with CHF were studied. Patients between the ages of 21 and 75 years with CHF > 3 months, stable symptoms compatible with NYHA (New York Heart Association) class II and III and left ventricular ejection fraction < 40 % were eligible for the study. Criteria for exclusion from the study were: systolic blood pressure < 90 mmHg or > 140 mmHg, HR (heart rate) < 50 or > 100 beats/min, therapy with long-acting nitrate preparations, history of intolerance of sildenafil, history of intolerance of ACE inhibitors, hospitalization for myocardial infarction, unstable angina or open heart surgery, TIA (transient ischaemic attack) or CVA (cerebrovascular attack) within 3 months, serum creatinine > 2.5 mg/dl or haemoglobin < 10 gm/dl. The protocol was approved by the Ethical Review Committee at Columbia Presbyterian Medical Center. All subjects gave written informed consent before participation.

**Brachial artery ultrasound imaging**

Endothelium-dependent FMD of the brachial artery was determined with high-resolution ultrasound imaging of the brachial artery with an 11 MHz linear array ultrasound probe connected to an ATL Apogee 800plus duplex ultrasound imaging machine (Advanced Technology Laboratories, Bothell, WA, U.S.A.) as described previously in detail [7]. All studies were performed by the same investigator and were read by a single investigator blinded to treatment assignment. Arterial diameter (cm) was determined as the internal dimension of the vessel wall, from trailing edge to leading edge of the anterior and posterior intimal markings from digitized images (NIH image software). Brachial artery diameter was measured at end-diastole at rest and 60 to 75 s after release of a forearm pneumatic cuff inflation to suprasystolic pressure for 5 min; five diameter measurements were averaged. FMD was determined as the percentage change in brachial artery diameter after cuff release compared with the resting brachial artery diameter. Brachial artery blood flow velocity was determined with a 1.2 mm pulsed Doppler ultrasound sampling volume placed in the centre of the image of the vessel lumen with internal software correction for the incident angle of 60°. Brachial artery blood flow velocity was measured at rest and immediately after release of a forearm pneumatic cuff inflation to suprasystolic pressure for 5 min. MBFV (mean blood flow velocity) was determined from hand tracings of the spectral display averaged over five beats.

**Neurohormonal measurements**

An indwelling catheter was placed into an antecubital vein for venous blood sampling in 41 of the 64 subjects (nine assigned to placebo, 11 assigned to ramipril alone, 12 assigned to sildenafil alone and nine assigned to sildenafil in combination with ramipril). Samples were not collected in 23 subjects due to technical problems with the intravenous catheter (n = 19) or subject refusal of the catheter (n = 4). A sample (5 ml) of blood was obtained from an indwelling catheter after 30-min rest in a supine position in a quiet darkened room and 4 h after study drug administration. Plasma was separated by cold centrifugation and stored at −80 °C. Plasma BNP (brain natriuretic peptide) was measured with a calibrated automated quantitative fluorescent sandwich immunoassay device (Bio-site® Diagnostic, San Diego, CA, U.S.A.) [14]. Plasma noradrenaline (norepinephrine) was measured with a HPLC method (ESA, Chelmsford, MA, U.S.A.) in the Yale Clinical Research Center Laboratory.

**Study design**

This was a prospective parallel group randomized comparison of the acute effects of the ACE inhibitor ramipril (10 mg), the selective PDE5 inhibitor sildenafil (50 mg), the combination of sildenafil and ramipril, and placebo on brachial artery FMD and neurohormone levels in ambulatory patients with CHF. Sildenafil (50 mg) and matching placebo were supplied by the manufacturer (Pfizer, New York, NY, U.S.A.). Ramipril (10 mg; Monarch Pharmaceuticals, Bristol, TN, U.S.A.) and matching placebo were purchased and prepared by the Columbia Presbyterian Medical Center Research Pharmacy. Subjects were randomly assigned in a double-blind manner to receive a single oral dose of sildenafil or placebo and a single oral dose of ramipril or placebo. Subjects were studied in the morning after an overnight fast. Background medication [other than ACE inhibitors and ARBs (angiotensin-receptor blockers)] was discontinued at least 12 h before the study, ACE inhibitors and ARBs were discontinued 72 h before the study. Brachial artery diameter and blood flow velocity were measured in the supine position at rest and after 5 min.
of transient arterial occlusion before and 1, 2 and 4 h after study drug administration. Blood for neurohormone measurements was obtained before and 4 h after study drug administration. Blood pressure and HR were determined before and 1, 2 and 4 h after study drug administration with an automated blood pressure monitor (Critikon, Tampa, FL, U.S.A.).

Data analysis

Serial measures of FMD, neurohormonal levels, blood pressure and HR over time were analysed using generalized linear models with adjustments of standard error estimates appropriate for the clustered data of the repeated measures design (Stata 8.0, College Park, TX, U.S.A.). Sixteen subjects per treatment group provided >80% power to detect a 2-fold interaction effect between sildenafil and ramipril on FMD assuming within subjects and residual standard deviations 150% of the mean and alpha = 0.05. Our initial models analysed the main effects of sildenafil compared with placebo and ramipril compared with placebo according to the factorial design of the study, but no significant interaction effect between sildenafil and ramipril was detected (P = 0.61). Accordingly, subsequent models utilized four treatment groups (placebo, ramipril alone, sildenafil alone and sildenafil in combination with ramipril) to analyse the treatment-by-time interaction. Plasma levels of noradrenaline and BNP were not normally distributed (by Shapiro-Wilk test), so the natural logarithmic transformations of these variables were used in regression models. Baseline characteristics were compared between treatment groups with one-way ANOVA or Fisher's Exact test as appropriate. For all comparisons, a P value < 0.05 was used to infer statistical significance.

RESULTS

Clinical characteristics

Clinical characteristics of the four treatment groups (50 mg of sildenafil, 10 mg of ramipril, combination of 50 mg of sildenafil and 10 mg of ramipril, and placebo) did not differ significantly (Table 1).

Brachial artery ultrasound measurements

Baseline measurements of brachial artery diameter, FMD and resting and post-ischaemic brachial artery MBFV before study drug administration did not differ significantly among the four treatment groups (Table 2). Ramipril alone significantly increased FMD at 4 h when compared with placebo (+2.3 ± 1.3%; P = 0.02; Table 2 and Figure 1). Sildenafil alone significantly increased FMD at 1, 2 and 4 h when compared with placebo (+3.5 ± 1.5, +4.5 ± 1.8 and +4.8 ± 1.3% respectively; all P < 0.03; Table 2 and Figure 1). Sildenafil in combination with ramipril significantly increased FMD at 1, 2 and 4 h when compared with placebo (+3.9 ± 1.4, +4.6 ± 1.8 and +3.7 ± 1.3% respectively; all P < 0.02; Table 2 and Figure 1). Sildenafil in combination with ramipril significantly increased FMD at 1, 2 and 4 h when compared with placebo (+3.5 ± 1.5, +4.5 ± 1.8 and +4.8 ± 1.3% respectively; all P < 0.03; Table 2 and Figure 1). FMD responses after sildenafil alone or
Table 2  Brachial artery ultrasound measurements before and at 1, 2 and 4 h after study drug administration in 64 subjects with CHF

BA, brachial artery. ∗P < 0.05 compared with placebo. Combination, ramipril + sildenafil.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rest BA diameter (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.38 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.37 ± 0.01</td>
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</tr>
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<td>Ramipril</td>
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<td>0.41 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>0.40 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>Combination</td>
<td>0.40 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td><strong>BA FMD (%)</strong></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>0.4 ± 0.4</td>
<td>0.1 ± 0.4</td>
<td>−0.2 ± 0.7</td>
<td>0.0 ± 0.4</td>
</tr>
<tr>
<td>Ramipril</td>
<td>0.9 ± 0.6</td>
<td>0.5 ± 0.8</td>
<td>0.7 ± 0.9</td>
<td>2.2 ± 0.5∗</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>1.4 ± 0.6</td>
<td>4.7 ± 1.4∗</td>
<td>4.3 ± 1.5∗</td>
<td>3.6 ± 1.2∗</td>
</tr>
<tr>
<td>Combination</td>
<td>0.9 ± 0.4</td>
<td>4.2 ± 1.1∗</td>
<td>4.3 ± 1.7∗</td>
<td>4.9 ± 1.2∗</td>
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<tr>
<td><strong>Rest BA MBFV (cm/s)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Placebo</td>
<td>5.6 ± 0.8</td>
<td>6.1 ± 0.8</td>
<td>6.0 ± 1.0</td>
<td>5.8 ± 0.8</td>
</tr>
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<td>Ramipril</td>
<td>5.7 ± 0.7</td>
<td>5.3 ± 0.7</td>
<td>5.1 ± 0.7</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>5.3 ± 0.6</td>
<td>6.2 ± 1.3</td>
<td>5.5 ± 0.6</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Combination</td>
<td>7.0 ± 1.0</td>
<td>5.7 ± 0.5</td>
<td>6.0 ± 0.7</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td><strong>Peak BA MBFV (cm/s)</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Placebo</td>
<td>53 ± 7</td>
<td>57 ± 6</td>
<td>51 ± 6</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>Ramipril</td>
<td>46 ± 7</td>
<td>43 ± 6</td>
<td>45 ± 6</td>
<td>40 ± 6</td>
</tr>
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<td>Sildenafil</td>
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<td>48 ± 4</td>
<td>53 ± 5</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>Combination</td>
<td>56 ± 5</td>
<td>47 ± 4</td>
<td>48 ± 5</td>
<td>43 ± 3</td>
</tr>
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</table>

Figure 1  Change from baseline FMD (ΔFMD) at 1, 2 and 4 h after administration of placebo (black bars), 10 mg of ramipril alone (dark-grey bars), 50 mg of sildenafil alone (light-grey bars) or the combination of sildenafil and ramipril (white bars)

Values are means ± S.E.M. ∗P < 0.05 compared with placebo.

sildenafil in combination with ramipril were significantly greater than FMD responses after ramipril alone with additive effects evident at the 4-h time point (Figure 1; P < 0.01 for both comparisons). Resting brachial artery diameter, resting brachial artery MBFV and peak brachial artery MBFV did not differ among treatment groups after study drug administration (Table 2).

**Blood pressure and HR measurements**

Baseline measurements of MAP (mean arterial pressure) and HR before study drug administration did not differ significantly among the four treatment groups (Table 3). Ramipril alone tended to decrease MAP at 1 h compared with placebo (−6 ± 5 mmHg; P = 0.056) and significantly decreased MAP at 2 and 4 h compared with placebo (−11 ± 5 and −12 ± 4 mmHg respectively; both P < 0.01; Table 3 and Figure 2). Sildenafil alone significantly decreased MAP at 1 h compared with placebo (−8 ± 5 mmHg; P = 0.039), but did not change MAP when compared with placebo at 2 and 4 h (Table 3 and Figure 2). Sildenafil alone significantly decreased MAP at 1 h compared with placebo (−8 ± 5 mmHg; P = 0.039), but did not change MAP when compared with placebo at 2 and 4 h (Table 3 and Figure 2). Sildenafil alone significantly decreased MAP at 1 h compared with placebo (−8 ± 5 mmHg; P = 0.039), but did not change MAP when compared with placebo at 2 and 4 h (Table 3 and Figure 2). 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Table 3  MAP and HR before and at 1, 2, and 4 h after study drug administration in 64 subjects with CHF

Values are means ± S.E.M. * P < 0.05 compared with placebo. Combination, ramipril + sildenafil.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Ramipril</td>
</tr>
<tr>
<td>0</td>
<td>94 ± 4</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>1</td>
<td>92 ± 5</td>
<td>86 ± 2*</td>
</tr>
<tr>
<td>2</td>
<td>92 ± 4</td>
<td>81 ± 2*</td>
</tr>
<tr>
<td>4</td>
<td>93 ± 4</td>
<td>81 ± 2*</td>
</tr>
</tbody>
</table>

Figure 2  Change from baseline MAP (ΔMAP) at 1, 2 and 4 h after administration of placebo (black bars), 10 mg of ramipril alone (dark-grey bars), 50 mg of sildenafil alone (light-grey bars) or the combination of sildenafil and ramipril (white bars)

Values are means ± S.E.M. * P < 0.05 compared with placebo.

**Neurohormone measurements**

The clinical characteristics and baseline flow-mediated vasodilation, MAP and HR of the 41 subjects with neurohormonal measurements did not differ significantly from those subjects without neurohormonal measurements. Before administration of the study drug, baseline plasma noradrenaline levels did not differ among the treatment groups (Table 4). Ramipril alone and sildenafil alone did not change plasma noradrenaline levels when compared with placebo (Table 4). Sildenafil in combination with ramipril increased plasma noradrenaline at 4 h compared with placebo (+167 ± 94 pg/ml; P < 0.01; Table 4). BNP levels did not differ among the four treatment groups at baseline or after study drug administration (Table 4).

Table 4  Plasma noradrenaline and BNP levels at baseline and 4 h after study drug administration in 41 subjects with CHF

Values are means ± S.E.M. (median). * P < 0.05 compared with placebo. Combination, ramipril + sildenafil. NE, noradrenaline.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Plasma NE (pg/ml)</th>
<th>Plasma BNP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Ramipril</td>
</tr>
<tr>
<td>0</td>
<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>1</td>
<td>Ramipril</td>
<td>Ramipril</td>
</tr>
<tr>
<td>2</td>
<td>Sildenafil</td>
<td>Sildenafil</td>
</tr>
<tr>
<td>4</td>
<td>Combination</td>
<td>Combination</td>
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</tbody>
</table>

**DISCUSSION**

The present findings demonstrate that both ramipril and sildenafil improved FMD when compared with placebo in patients with CHF, with additive but not synergistic effects evident at the 4 h FMD measurement. These findings suggest that ACE inhibition and PDE5 inhibition increase FMD by independent mechanisms and provide additive benefits on endothelial function in patients with CHF.

**Effects of ramipril on FMD**

Ramipril is an ACE inhibitor with high tissue affinity that is rapidly absorbed and metabolized with peak plasma levels of the active metabolite, ramiprilat, reported at 2 h after oral administration [15]. To our knowledge, the present study is the first report of the acute effects of oral administration of the ACE inhibitor ramipril on FMD in the brachial artery of subjects with CHF. Ramipril has been shown previously to enhance endothelial function in isolated aortic and pulmonary artery rings in experimental models of endothelial dysfunction and heart failure and to improve radial artery FMD in subjects with coronary artery disease [16–18]. In small clinical CHF studies, acute intra-arterial administration of quinaprilat and 8 weeks of oral administration of perindopril both improved radial artery FMD [8,9]. Blood-pressure-lowering effects of ramipril were comparable with those reported in a previous haemodynamic study in ACE-inhibitor-naïve CHF subjects [19].

**Effects of sildenafil on FMD**

Sildenafil is a selective PDE5 inhibitor used clinically for the treatment of erectile dysfunction ([20], but see [20a]). The beneficial effects of sildenafil on erectile function are attributable to potentiation of cGMP signalling.
in corpus cavernosum smooth muscle tissue [21–23]. The current finding of improved FMD after administration of sildenafil is in accordance with experimental studies in isolated vascular tissues and previous clinical studies in patients with endothelial dysfunction in association with CHF, coronary artery disease and diabetes mellitus [7,24,25]. The observed time course of the effects of sildenafil on FMD in our population are consistent with its previously characterized pharmacokinetics [26]. Blood-pressure-lowering effects of sildenafil were comparable with those reported in previous studies of patients with heart failure and hypertension [10,27–29].

**Possible mechanisms**

No prior experimental or clinical studies on the specific interaction between ACE inhibition and PDE5 inhibition have been reported. ACE inhibitors have been reported to improve endothelium-dependent vasodilation by increasing production of NO and decreasing degradation of NO by reactive oxygen species [4,11,13,30]. ACE inhibitors may also have direct and indirect effects on the effector response to NO signalling in vascular smooth muscle. Angiotensin II decreases soluble guanylate cyclase expression and activity and increases degradation of cGMP by activation of PDE1 and/or PDE5 in vascular smooth muscle [11,12,31–34]. Based on these previous observations, we hypothesized that ramipril and sildenafil would increase FMD by a synergistic cGMP-dependent mechanism. Our unanticipated finding of no significant interaction between these two agents on FMD and blood pressure suggests that responses to these agents are attributable to independent mechanisms of action. The effects of sildenafil are almost certainly attributable to potentiation of cGMP signalling, as PDE5 inhibitors induce vasodilatation only in the presence of NO [35,36]. Accordingly, our present findings suggest that the acute effects of ramipril on FMD are not attributable to augmentation of cGMP signalling in vascular smooth muscle. The absence of synergistic effects on blood pressure is consistent with this interpretation [10,29,37,38]. This interpretation is also consistent with a previous observation that increased FMD in patients with coronary artery disease in response to chronic treatment with the ACE inhibitor quinapril is not associated with increased NO production [39]. ACE inhibition may acutely increase FMD by a prostaglandin-mediated cAMP-dependent mechanism or by kinin-mediated release of endothelium-derived hyperpolarizing factor [13,40,41].

**Neurohormonal effects**

Although both agents significantly decreased MAP when compared with placebo, neither ramipril alone nor sildenafil alone were associated with increased plasma noradrenaline levels. These observations are consistent with previous studies in CHF patients [27]. The combination of sildenafil and ramipril did not decrease blood pressure further when compared with ramipril alone, yet was associated with a small, but statistically significant, increase in plasma noradrenaline levels when compared with placebo. The clinical importance of this finding is uncertain, since the combination of ramipril and sildenafil significantly decreased HR when compared with placebo. Our findings suggest that combination therapy may have mixed autonomic effects independent of blood pressure changes [42].

**Clinical implications**

Endothelial dysfunction impairs skeletal muscle blood flow during exercise and is associated with greater impairment of functional capacity in CHF patients [43,44]. Decreased endothelium-dependent vasodilation in CHF is attributable to decreased activity of the L-arginine/NO metabolic pathway, decreased bioavailability of NO and decreased response to NO in vascular smooth muscle [2,4–6,45]. Since the magnitude of the effects of sildenafil on FMD were significantly greater than those of ramipril, our present findings suggest that abnormalities in cGMP signalling in vascular smooth muscle may be the predominant determinant of impaired NO-mediated vasodilation in CHF. Sildenafil has been reported to be associated with acute increases in exercise capacity in patients with CHF [27]. Since acute pharmacological effects may not be predictive of long-term benefits during chronic therapy, additional studies to investigate the long-term interaction of these two classes of agents are warranted.

**Study limitations**

Our observations are limited to the study of the acute interaction between ramipril and sildenafil in CHF subjects and may not be relevant to chronic treatment settings, other cardiovascular disease populations or other agents of the same pharmacological classes. Since our present study sample was small and clinically heterogeneous, additional studies in larger populations are needed to confirm our findings. Chronic therapy with ACE inhibitors or ARBs was stopped for 72 h before the study. Although this drug-free interval was sufficient to observe a large decrease in blood pressure after ramipril dosing comparable with a previous report in ACE-inhibitor-naïve patients, we cannot exclude the possibility that lingering tissue effects may have contributed to our findings. Other background medication, although stopped 12 h before the present study, may also have contributed to our findings. Although the cellular mechanisms contributing to our findings cannot be fully discerned in this clinical investigation, the physiological responses observed demonstrate that these two classes of agents may be useful together to provide additive improvement of endothelial function in CHF subjects.

In conclusion, acute administration of ramipril and sildenafil increased FMD in the brachial artery of subjects...
with CHF. The lack of a significant interaction between these two agents suggests that beneficial effects on FMD are mediated by independent mechanisms. Combined therapy may provide additive benefits on endothelial function in patients with CHF and other cardiovascular disease states.

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