Structure and in vitro function of human subcutaneous small arteries in mild heart failure

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Twenty-seven patients with clinically diagnosed congestive heart failure (Killip classes II and III), after an acute myocardial infarction, were studied. The patients were a subset of those recruited at Leeds, UK, as part of the Acute Infarction Ramipril Efficacy (AIRÉ) Study (3), and all patients displayed clinical evidence of heart failure, which was defined as at least one of the following: evidence of left ventricular failure, evidence of pulmonary edema, or auscultatory evidence of a third heart sound with persistent tachycardia (3). In accordance with the AIRÉ Study entry criteria, patients with severe heart failure (New York Heart Association classification grade IV) had been excluded from the study. Patients were randomly assigned to therapy with placebo or ramipril (2.5–5 mg twice daily) starting 3–10 days after myocardial infarction. The mean duration of treatment was 27 ± 2 mo (range 6–43 mo). Standard therapy was maintained for all patients throughout the trial, and concomitant medications are indicated in Table 1. Subcutaneous skin biopsies were performed on patients at the close of the trial (see below). At this time, 24 of the 27 patients studied had a history of overt congestive heart failure that required treatment with diuretics or inotropic drugs and/or vasodilators for symptomatic relief, fulfilling the Studies of Left Ventricular Dysfunction (SOLVD) criteria for congestive heart failure (39). Three of the patients fulfilled the SOLVD criteria for asymptomatic left ventricular dysfunction. All patients had evidence of reduced left ventricular function on the basis of radionuclide scanning (method B) (24) at the time of biopsy. Ten healthy volunteers from the general public were studied as control subjects. They had no medical history of cardiovascular disease, and systolic blood pressure was measured to be <140 mmHg.

THE PREVALENCE OF HEART failure in the population is between 1 and 3%, carrying with it a particularly poor prognosis. Although the primary abnormality is loss of functioning myocardium, the resulting fall in cardiac output leads to an activation of a number of compensatory neuroendocrine mechanisms, such as the sympathetic nervous and renin-angiotensin systems (8, 30, 36, 37). To maintain blood pressure, these mechanisms produce inotropic stimulation of the residual myocardium, peripheral vasoconstriction, and fluid retention. Although in the short term cardiac output is improved, over a longer period direct cardiotoxic effects of angiotensin II and norepinephrine and an increase in peripheral vascular resistance contribute to the progressive decline in cardiac function.

The role of the peripheral vasculature in this process is not clear. In theory, high concentrations of catecholamines and plasma renin (18, 25, 32, 41), through the formation of angiotensin II, could influence the function of small blood vessels, and also there are reports that the disease process itself is associated with impairment of endothelium-dependent relaxation (5, 11, 26, 28). In addition, there is the possibility that sympathomimetic amines and angiotensin II could cause structural changes in the vasculature similar to those seen in hypertensive subjects. Indeed, a study of patients with heart failure with low-to-normal blood pressure might help resolve the debate over the importance of pressure per se or trophic hormone influence as major determinants of small vessel hypertrophy.

In vitro investigation of the properties of peripheral resistance arteries in heart failure has been limited to one study of a small group of patients receiving a variety of medications (5). Therefore, we decided to examine the structure and function of isolated, peripheral small arteries from patients treated for heart failure after myocardial infarction. It was possible to perform this study on a subpopulation of patients randomized to placebo or the angiotensin-converting enzyme (ACE) inhibitor ramipril, in addition to conventional therapy for heart failure, thereby allowing an analysis of the effects of this class of drug on small artery structure and function in this clinical situation.

METHODS

Patients and Control Subjects

Twenty-seven patients with clinically diagnosed congestive heart failure (Killip classes II and III), after an acute myocardial infarction, were studied. The patients were a subset of those recruited at Leeds, UK, as part of the Acute Infarction Ramipril Efficacy (AIRÉ) Study (3), and all patients displayed clinical evidence of heart failure, which was defined as at least one of the following: evidence of left ventricular failure, evidence of pulmonary edema, or auscultatory evidence of a third heart sound with persistent tachycardia (3). In accordance with the AIRÉ Study entry criteria, patients with severe heart failure (New York Heart Association classification grade IV) had been excluded from the study. Patients were randomly assigned to therapy with placebo or ramipril (2.5–5 mg twice daily) starting 3–10 days after myocardial infarction. The mean duration of treatment was 27 ± 2 mo (range 6–43 mo). Standard therapy was maintained for all patients throughout the trial, and concomitant medications are indicated in Table 1. Subcutaneous skin biopsies were performed on patients at the close of the trial (see below). At this time, 24 of the 27 patients studied had a history of overt congestive heart failure that required treatment with diuretics or inotropic drugs and/or vasodilators for symptomatic relief, fulfilling the Studies of Left Ventricular Dysfunction (SOLVD) criteria for congestive heart failure (39). Three of the patients fulfilled the SOLVD criteria for asymptomatic left ventricular dysfunction. All patients had evidence of reduced left ventricular function on the basis of radionuclide scanning (method B) (24) at the time of biopsy. Ten healthy volunteers from the general public were studied as control subjects. They had no medical history of cardiovascular disease, and systolic blood pressure was measured to be <140 mmHg.

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Table 1. Patient concomitant medication

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Placebo (n = 15)</th>
<th>Ramipril (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>11 (73)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>3 (20)</td>
<td>2 (16)</td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>5 (33)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>5 (33)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>9 (60)</td>
<td>8 (67)</td>
</tr>
</tbody>
</table>

Values represent number of patients receiving concomitant medication in groups randomized to therapy with placebo or ramipril, with percentages in parentheses.

disease or clinical evidence of heart failure or other cardiac pathology. All patients/subjects gave informed, written consent to participate in the study, and the protocol was approved by the local ethics committee.

Biopsy Procedure and Artery Preparation

By use of the method described previously (1, 2), gluteal skin biopsies were obtained under local anesthetic (2% lignocaine) and placed in cold physiological salt solution (PSS, see Drugs and Solutions). Drug therapy was withheld for 24 h before the biopsy was performed. Subcutaneous small arteries (~250 µm ID, 2-mm segment length) were dissected from the biopsy, cleaned of adherent fat and connective tissue, and mounted on a wire myograph (JP Trading, Aarhus, Denmark) for measurement of morphology and isometric tension (22). In most cases, two vessels from each biopsy were studied. Myograph-mounted vessels were incubated in PSS, warmed to 37°C, gassed with oxygen containing 5% carbon dioxide, and allowed to equilibrate for ~30 min.

Experimental Protocol

Artery wall morphology was measured using a ×40 magnification saline immersion lens (Zeiss), as described previously (35). The resting tension–internal circumference relation was determined for each artery. Vessels were set to a normalized internal circumference of 0.9L100, where L100 is the internal circumference of the vessel under a transmural pressure of 100 mmHg (35). Effective normalized luminal diameters (IL) were calculated as 0.9L100/D.

In a standard start procedure, arteries were activated with 5 µmol/l norepinephrine on three occasions, each for a period of 2 min with a 5-min washout between activations. Contraction elicited by norepinephrine was expressed as effective active pressure (ΔP) on the basis of the law of Laplace, where ΔP = 2ΔT/L and ΔT is the change in tension. Arteries unable to produce an effective active pressure >12 kPa were considered unviable and were not used in further analysis. With use of this criterion, 8 of a total of 66 arteries were rejected spanning the subject/patient groups. Cumulative concentration-response curves were performed to the vasoconstrictor agonists norepinephrine (0.01–10 µmol/l), in the absence and presence of cocaine (3 µmol/l) and angiotensin II (0.0001–1 µmol/l) and to the vasodilator agonists acetylcholine (0.001–10 µmol/l) and sodium nitroprusside (0.001–10 µmol/l) after preconstriction with 5 µmol/l norepinephrine. Vessels were stimulated for 2 min at each concentration of a drug. Concentration-response curves were separated by a 20-min period, during which time arteries were incubated in PSS or, where appropriate, cocaine. The order in which the concentration–response curves were performed was randomized.

Finally, a frequency-response curve to electrical field stimulation was performed to determine the sensitivity to endogenous neurotransmitter released from intramuscular sympathetic nerves. Arteries were incubated in PSS throughout the stimulation. Platinum foil electrodes were secured in the plastic myograph mounting heads and connected to an electrical stimulator (Harvard Apparatus, Kent, UK), as described previously (40). Arteries were stimulated at 20 V, 0.2-ms pulse width, 20-s pulse train, with a 5-min interval between trains, over a frequency range of 1–24 Hz. Previous experiments indicate that vasoconstriction elicited in this manner is sensitive to the nerve toxin tetrodotoxin and is mediated via α1- and α2-adrenergic receptors (40).

Data Analysis

Values are means ± SE; n refers to the number of biopsies studied. Where two artery segments were studied from a biopsy, the data from the vessels were averaged to provide a single value per biopsy. Concentration- and frequency-response curves were analyzed by repeated-measures ANOVA. This analysis was carried out on the raw data by the method of maximum likelihood using the GLIM 3-77 package (4), and the adequacy of these analyses was determined by constructing normal probability plots in conjunction with the Filliben correlation coefficient (14). Tukey’s multiple comparison test (44) was used to determine differences between the three subject/patient groups (control, placebo, ramipril) and the effect of cocaine on norepinephrine sensitivity. Norepinephrine pD2 values were calculated as −log ED50, where ED50 was the agonist concentration required to elicit half-maximal contraction. One-way ANOVA followed by Dunnett’s test for multiple comparisons was used to compare demographic, morphological, maximum response, and pD2 values between control subjects and heart failure patients treated with placebo and between patients treated with ramipril and placebo. Statistical significance was set at the conventional 5% level.

Drugs and Solutions

PSS had the following composition (mmol/l): 119 NaCl, 4.7 KCl, 2.5 CaCl2, 1.17 MgSO4, 1.18 KH2PO4, 0.026 K2EDTA, and 5.5 D-glucose. (±)-Norepinephrine hydrochloride, acetylcholine hydrochloride, and sodium nitroprusside were obtained from Sigma Chemical.

RESULTS

Demographic Data

Demographic details, taken at the time of biopsy, of control subjects and heart failure patients treated with placebo or ramipril are shown in Table 2. There was no significant difference in the age of subjects or patients in the three groups. Predictably, systolic blood pressure was significantly higher in control subjects than in heart failure patients treated with placebo (P < 0.001). Systolic blood pressure was also higher in patients treated with placebo than in those treated with ramipril, but the difference was not statistically significant. Diastolic blood pressure was similar in control subjects and patients treated with placebo but was significantly lower in patients treated with ramipril than in patients treated with placebo (P < 0.001). Left ventricular ejection fraction (LVEF) was 47 ± 3% for patients treated with placebo and 41 ± 4% for patients treated with ramipril; there was no significant difference between the two treatment groups.
Table 2. Demographic data

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 10)</th>
<th>Heart Failure Patients</th>
<th>One-Way ANOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 15)</td>
<td>Ramipril (n = 12)</td>
<td></td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>8:2</td>
<td>13:2</td>
<td>ND</td>
</tr>
<tr>
<td>Age, yr</td>
<td>61 ± 2</td>
<td>66 ± 2</td>
<td>F = 1.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66 ± 2</td>
<td>P = 0.22</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>149 ± 3*</td>
<td>129 ± 3</td>
<td>F = 12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>119 ± 5</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>80 ± 4</td>
<td>80 ± 2</td>
<td>F = 5.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71 ± 2*</td>
<td>P = 0.009</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>ND</td>
<td>47 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>41 ± 4</td>
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</tbody>
</table>

Values are means ± SE of data collected at the time of biopsy. Blood pressures represent single measurements. SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, radionuclide left ventricular ejection fraction [normal range 64% (19)]; ND, not determined. One-way ANOVA test statistics are presented as F ratios and P values. *P < 0.001 vs. placebo (Dunnett’s test).

Morphology

The morphology data for arteries from each of the subject/patient groups are shown in Table 3. One-way ANOVA failed to reveal significant differences among the three groups for normalized luminal diameter, media thickness, media-to-lumen ratio, or media cross-sectional area. Therefore, there were no significant differences in any parameter between arteries from control subjects and patients receiving placebo. However, luminal diameter tended to be greater and media-to-lumen ratio tended to be less in arteries from ramipril-treated patients than in arteries from placebo. A multiple comparison test (Tukey) between the ramipril-treated group and the placebo and control subject/patient groups are shown in Table 3. One-way ANOVA test statistics are presented as F ratios and P values.

Vasoconstrictor Responses

Angiotensin II elicited vasoconstriction, which was similar in arteries from control subjects and heart failure patients treated with placebo but was significantly enhanced in patients treated with ramipril (Fig. 1; P < 0.001). The maximum response to norepinephrine (5 µM), expressed as effective active pressure, was not significantly increased in arteries from control subjects (21 ± 4 kPa) and patients treated with placebo (20 ± 2 kPa) or ramipril (21 ± 2 kPa). The sensitivity to norepinephrine was similar in arteries from control subjects and patients treated with placebo but was significantly enhanced in patients treated with ramipril (Fig. 2; P < 0.001). Norepinephrine pD2 values are given in Table 4. Incubation of arteries with cocaine, to block neuronal amine uptake, significantly increased sensitivity to norepinephrine overall (P < 0.001); the effect of cocaine was similar in all subject/patient groups (Table 4).

The maximum response to electrical field stimulation (25 Hz), expressed as effective active pressure, was not

Table 3. Morphology data

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 10)</th>
<th>Heart Failure Patients</th>
<th>One-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 14)</td>
<td>Ramipril (n = 12)</td>
<td></td>
</tr>
<tr>
<td>Lumen, µm</td>
<td>242±19</td>
<td>212±7</td>
<td>F = 2.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>248±13</td>
<td>P = 0.098</td>
</tr>
<tr>
<td>Media, µm</td>
<td>16.8±0.9</td>
<td>14.6±1.2</td>
<td>F = 1.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.4±1.2</td>
<td>P = 0.16</td>
</tr>
<tr>
<td>Media-to-lumen ratio, %</td>
<td>7.8±0.9</td>
<td>7.3±0.7</td>
<td>F = 2.81</td>
</tr>
<tr>
<td>Media CSA, µm²</td>
<td>13,790±1,200</td>
<td>10,390±780</td>
<td>F = 1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12,320±1,870</td>
<td>P = 0.22</td>
</tr>
</tbody>
</table>

Values are means ± SE for normalized arteries. CSA, cross-sectional area. One-way ANOVA test statistics are presented as F ratios and P values.
significantly different in arteries from control subjects (6.0 ± 0.6 kPa) and patients treated with placebo (4.5 ± 0.6 kPa) but was significantly greater in arteries from patients treated with ramipril (7.0 ± 0.8 kPa) than in placebo-treated patients (P < 0.05). Frequency-response curves to electrical field stimulation are shown in Fig. 3. The sensitivity to electrical field stimulation was not significantly different in arteries from control subjects or heart failure patients treated with placebo or ramipril (Fig. 3).

Vasodilator Responses

Repeated-measures ANOVA failed to reveal a significant difference in acetylcholine-induced relaxation in arteries from the three subject/patient groups (Fig. 4). However, examination of the 95% confidence limits shows a significant difference between arteries from patients treated with ramipril and arteries from patients treated with placebo at the three highest acetylcholine concentrations (1, 3, and 10 µmol/l, P < 0.05). Relaxation to sodium nitroprusside was not significantly different in arteries from control subjects or heart failure patients treated with placebo or ramipril (Fig. 3).

Table 4. Norepinephrine sensitivity

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 9)</th>
<th>Heart Failure Patients</th>
<th>One-Way ANOVA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Placebo (n = 14)</td>
<td>Ramipril (n = 12)</td>
<td></td>
</tr>
<tr>
<td>pD2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>6.16 ± 0.12</td>
<td>6.20 ± 0.10</td>
<td>F = 5.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.60 ± 0.08*</td>
<td>P = 0.009</td>
</tr>
<tr>
<td>NE-Coc</td>
<td>6.49 ± 0.10</td>
<td>6.50 ± 0.13</td>
<td>F = 5.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.99 ± 0.10*</td>
<td>P = 0.011</td>
</tr>
<tr>
<td>ΔpD2</td>
<td>0.33</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.39</td>
<td></td>
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</tbody>
</table>

Values are means ± SE expressed as pD2 (i.e., −log ED50, where ED50 is agonist concentration required to elicit half-maximum contraction). NE, norepinephrine; Coc, cocaine; ΔpD2, change in pD2 due to cocaine. One-way ANOVA test statistics are presented as F ratios and P values. *P < 0.001 vs. placebo (Dunnett’s test).

Fig. 4. Concentration-response curves to acetylcholine in arteries from control subjects (○, n = 9) and patients treated with placebo (□, n = 14) or ramipril (■, n = 12). Responses (means ± SE) are expressed as percentage of norepinephrine-induced preconstriction. Acetylcholine-induced relaxation in arteries from control subjects was not significantly different from that in placebo-treated patients but was significantly reduced in arteries from patients treated with ramipril compared with those treated with placebo at the 3 highest concentrations (P < 0.05).

DISCUSSION

In the present study we examined isolated segments of subcutaneous small arteries from patients with clinical evidence of heart failure after acute myocardial infarction treated with the ACE inhibitor ramipril or placebo and matched healthy control subjects. Patients had been recruited as part of the AIRE Study (3) and met the AIRE Study criteria for diagnosis of heart failure after myocardial infarction. Patients with se-
vere heart failure (New York Heart Association classification grade IV) were excluded from the study, restricting the scope of this investigation to mild heart failure. At the time of investigation (6‐43 mo after myocardial infarction) the patients displayed symptomatic mild heart failure on the basis of the SOLVD criteria (39) and demonstrated impaired left ventricular function on the basis of radionuclide scanning. The results suggest that there is little alteration in vascular structure, contraction, or relaxation in subcutaneous small arteries from patients with mild-to-moderate heart failure who were treated with placebo. However, ACE inhibitor therapy tended to modify vascular structure and significantly influenced vascular function in subcutaneous arteries obtained from patients with mild heart failure.

There were no significant differences in vascular morphology between subcutaneous small arteries from mild heart failure patients and those from healthy control subjects. However, normalized luminal diameter tended to be increased and media-to-lumen ratio decreased in arteries from patients receiving ramipril therapy compared with placebo. These differences may indicate an influence of ACE inhibitors on vascular structure. The cross-sectional area of media, which is an index of the amount of smooth muscle, was not reduced in arteries from patients receiving ramipril. Therefore, such vascular alterations may represent a rearrangement of preexisting smooth muscle around a larger lumen, i.e., “reverse remodeling” (19), or an increase in the elasticity of the vessels so that a given transmural pressure is able to distend arteries to a greater diameter. Evidence in the literature suggests that ACE inhibitors increase vascular compliance (10, 16, 33, 38, 42), offering a possible explanation for the apparent increase in luminal diameter and reduction in media-to-lumen ratio in arteries from patients treated with ramipril.

We observed little difference in the function of subcutaneous small arteries between placebo-treated patients with mild heart failure and control subjects. Responses to norepinephrine, angiotensin II, and electrical field stimulation were not significantly different in terms of maximum contraction and sensitivity in arteries from these two groups. These data suggest little alteration in the vascular contraction of subcutaneous small arteries in response to exogenous agonists or endogenously released neurotransmitter in patients with clinical evidence of mild heart failure treated with placebo. Our findings contrast with those of another study (5), in which subcutaneous arteries from six patients with longstanding (≥5–10 yr) and more severe heart failure (28 ± 6% LVEF), whose treatment included ACE inhibitors, were studied. In the earlier study the maximum contractility to a number of constrictor stimuli, including norepinephrine and angiotensin II, was significantly reduced in arteries from the patients (5). This was considered by the authors to be a nonspecific “fatiguing” of the vascular smooth muscle (5). The difference in findings may reflect the longstanding and more severe nature of the disease in the patients studied by Angus and colleagues (5) than in our patients with milder disease. However, although the present study differed from that of Angus and colleagues, in that we were able to distinguish between patients receiving ACE inhibitors and those who were not, neither study provides any evidence for reduced vascular smooth muscle sensitivity to norepinephrine or angiotensin II in arteries from patients with mild or more severe heart failure.

The vasoconstrictor response to angiotensin II and norepinephrine was augmented in subcutaneous small arteries from patients treated with ramipril, possibly reflecting upregulation of receptor-mediated events in response to decreased concentrations of these hormones at the level of the receptor. Measurement of plasma angiotensin II indicates that circulating levels are reduced as a consequence of ACE inhibitor therapy, although not necessarily throughout 24 h, depending on dosing schedule and dose and type (long or short acting) of agent (34). However, if this were correct, one might expect downregulation by higher levels of angiotensin II in patients with congestive heart failure treated with placebo than in control subjects, which we did not observe. In addition, there is evidence to suggest that ACE inhibitors reduce levels of plasma norepinephrine (6, 15, 43). This may result from a diminished stimulation of presynaptic angiotensin II receptors at sympathetic neuroeffector junctions, which normally serve to facilitate norepinephrine overflow (29). In addition, ACE inhibitors may reduce plasma norepinephrine by other effects, such as improved central hemodynamics, central nervous system effects, and lowering of sympathetic outflow. Therefore, the increased vasoconstrictor response to angiotensin II and norepinephrine after ACE inhibitor therapy may reflect an interaction between the two systems, possibly at the neuroeffector junction. Although the sensitivity to electrical field stimulation was not significantly different in arteries from control subjects and patients with mild heart failure in either treatment group, the magnitude of the response to nerve stimulation was increased in arteries from patients receiving ramipril compared with placebo, which may also reflect an upregulation of the postjunctional response to norepinephrine.

In the present study, subcutaneous small arteries from placebo-treated patients with mild heart failure demonstrated normal endothelium-dependent and independent relaxation. Again, these findings were in contrast to those from patients with longstanding and more severe heart failure, in which endothelium-dependent relaxation was markedly impaired in isolated subcutaneous arteries obtained from the same region (5). Furthermore, in vivo studies in patients with heart failure (mean LVEF <30%) suggest that agonist-induced, endothelium-dependent vasodilation is impaired in the forearm (11, 15) and femoral (14, 38) circulations, and these impairments could not be attributed to altered plasma lipids or other risk factors. However, the response to dilators that act directly on vascular smooth muscle (endothelium independent) is also impaired in heart failure (21, 23, 26, 27, 31), so
dysfunction may reside in part with the vascular smooth muscle itself. Thus the impairment of dilator function may reflect the severity of the disease; although relaxation is impaired in patients with severe or longstanding heart failure, it is not readily apparent in our patients with milder disease. In this respect, the present data may support a previous observation that dilator function was preserved in a subgroup of patients with significantly higher LVEF (26).

Although endothelium-dependent relaxation was not impaired in subcutaneous arteries from patients with mild heart failure treated with placebo, ramipril therapy was associated with a slight reduction in maximum relaxation compared with arteries from placebo-treated patients. The reason for this is unclear, particularly in view of reports which suggest that ACE inhibitors improve endothelium-dependent relaxation in animal models of hypertension (7, 22) and essential hypertension (20), where endothelial function is abnormal in the first instance. However, ACE inhibitors had no effect on endothelium-dependent relaxation in control animals, which demonstrated normal relaxation. Thus the reason for a slight reduction in relaxation in subcutaneous arteries from patients treated with ramipril remains to be resolved.

Limitations of the Study

Methodology. This study was performed using in vitro wire myography. Although this technique has achieved a wide degree of acceptability, concerns have been expressed that the wires distort the shape of the blood vessel and may damage the endothelium. In addition, there is no flow through the lumen of the artery, and exogenously applied hormones, drugs, and antagonists are administered to the adventitial surface of the vessel. Consequently, investigators have begun to use pressure myography, which necessitates the mounting of similarly sized segments of small arteries on cannulas; this method maintains the physiological contour of the vessel, does not traumatize the endothelial surface, and permits the presentation of hormones and drugs to the luminal surface. Therefore, there is the possibility that the methodology we used might have influenced the outcome. With respect to morphology, we have shown that pressure and wire myography provide largely similar results, and so we are confident that this is not a problem (12, 13). With regard to the functional findings, relaxation of small arteries to acetylcholine is similar with use of either method, whereas sensitivity to norepinephrine is greater in pressurized vessels. However, there is no evidence that methodology produces different changes in study populations (12, 21). In summary, we are confident that the in vitro methods employed have not influenced our findings.

Control subjects. To make comparisons with our patient groups, we selected similarly aged and gender-matched subjects with no history or evidence of cardiovascular disease. These individuals had a significantly higher average systolic blood pressure but diastolic pressures comparable to our patient groups. Therefore, it is possible that we selected previously undiagnosed hypertensive individuals into the control group. In doing so we might have increased the arterial morphology parameters and masked a change in heart failure patients. We believe this is unlikely for the following reasons: 1) we would anticipate that further readings of blood pressure over a protracted period would show a decline in the individual values, and 2) the morphology readings we obtained are very close to normotensive values that we have previously published (9, 27). Therefore, we are confident that a morphological abnormality in heart failure has not been missed.

Drug therapy for heart failure. All our patients with heart failure were receiving a number of drugs in addition to ramipril or placebo. It is possible that these agents might have influenced our findings, but the distribution of such drugs was comparable between the study groups, and we consider this unlikely.

Sample size and duration of follow-up. It has to be conceded that the size of our study population is small. Consequently, only mild and variable changes in arterial structure and function might have been missed. This is entirely possible, although Angus et al. (5) did find a functional change in their smaller cohort of patients with severe heart failure, and our morphological findings are in accord with our previously published data on normotensive subjects (17, 27). Certainly, these in vitro studies with their limited assessment of function may miss in vivo abnormalities. Larger cohorts of patients would permit a more comprehensive series of functional tests to be carried out. With regard to the duration of follow-up, this varied widely (6–43 mo). Although this was the same overall for both groups of patients with congestive heart failure, the small numbers make it impossible to examine whether structure and function change with time. A reinspection of the fate of our patients after 3 yr has revealed just one death in the placebo-treated group (17).

In conclusion, subcutaneous small arteries from placebo-treated patients with clinical evidence of mild heart failure demonstrated no significant differences in vascular morphology, constriction, or relaxation compared with vessels from normal control subjects. Subcutaneous arteries were studied, since they are obtained from a vascular bed that is readily accessible in patients and healthy control subjects, although it is not clear how representative they are of the peripheral vasculature as a whole. Treatment of patients with mild heart failure with an ACE inhibitor significantly reduced diastolic blood pressure and tended to increase arterial luminal diameter and reduce media-to-lumen ratio. Our results have shown that ACE inhibitor therapy was associated with an augmented constrictor response to angiotensin II and norepinephrine in subcutaneous small arteries, which may reflect upregulation of these receptor-mediated events as a result of diminished levels of these hormones in vivo. The parallel change in constrictor responses mediated by angiotensin II and α-adrenergic receptors after ACE inhibition supports the existence of a functional interaction between these two systems in humans.
REFERENCES


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