Endothelium-dependent vasodilatation is not selectively impaired in patients with chronic heart failure secondary to valvular heart disease and congenital heart disease

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This study examined possible selective impairment of endothelial dysfunction in the peripheral vascular bed in patients with chronic heart failure in the absence of confounding factors influencing endothelial function (i.e. hypertension, hypercholesterolaemia and diabetes mellitus). Several recent studies have suggested that endothelium-dependent peripheral vasodilation is impaired but endothelium-independent vasodilation is preserved in patients with chronic heart failure. However, a classical paper has demonstrated that sodium nitrite-mediated calf blood flow is clearly depressed in patients with valvular heart disease and cardiomyopathy. We examined forearm blood flow changes mediated by acetylcholine and nitroprusside in patients with valvular heart disease (n=55) or congenital heart disease (n=13), and a comparison was made with healthy volunteers (n=21). The blood flow changes mediated by acetylcholine and nitroprusside were significantly impaired in both patient groups (P<0.01). When blood flow responses were collected from all patients, two types of vasodilatory capacity were found to have decreased significantly with increasing clinical severity of heart failure (New York Heart Association functional class; P<0.01). This suggests that the peripheral vasodilatory responses mediated by endothelium-dependent and endothelium-independent vasodilators are significantly impaired in patients with symptomatic chronic heart failure due to non-ischaemic heart disease.

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Key Words: Endothelium, smooth muscle, heart failure, valvular heart disease, congenital heart disease, peripheral circulation.

Introduction

As chronic heart failure progresses, there appears to be a concomitant increase in peripheral resistance and a decrease in limb blood flow. These abnormalities of peripheral circulation have been thought to result from stimulation of the neuroendocrine system and dysregulation of local vasoactive substances, and to play an important role in the pathogenesis and maintenance of heart failure[1,2].

Several recent studies have shown that endothelial dysfunction as evaluated by cholinergic stimuli (i.e. acetylcholine, metacholine) develops in peripheral resistance vessels in patients with heart failure[3-9]. Some of these studies have demonstrated that endothelium-independent vasorelaxation mediated by nitrovasodilators is preserved[3,4]. These findings suggest that endothelium-dependent vasorelaxation is selectively attenuated in the peripheral vascular bed in patients with congestive heart failure. However, a classical study examining patients with heart failure due to valvular heart disease and cardiomyopathy showed a clear reduction in calf blood flow induced by femoral arterial infusion of sodium nitrite[6].

The response of endothelium-dependent forearm blood flow to acetylcholine has been reported to be impaired in patients with hypercholesterolaemia, glucose intolerance and hypertension[7-10]. Blood flow response to cholinergic stimuli would thus be biased in cases where these disorders are complicated by chronic heart failure.

The aim of this study was to determine whether endothelium-dependent vasorelaxation is selectively depressed in heart failure patients in the absence of these confounding factors. Because these disorders are frequent complications of chronic heart failure due to coronary heart disease, only those patients who were free from these complications and whose chronic heart...
failure was due to valvular heart disease or congenital heart disease were recruited for the study.

The changes in regional blood flow induced by intra-arterial infusion of acetylcholine and nitroprusside were measured by forearm plethysmography, and the responses obtained were compared to those obtained from healthy age- and sex-matched controls.

Methods

Subjects

A total of 68 patients with chronic heart failure were recruited for this study. The aetiology of chronic heart failure was valvular heart disease in 55 patients and congenital heart disease in 13. Twenty-one healthy volunteers were enrolled as a control group after examination by routine blood tests, electrocardiogram and chest X-ray. None of these subjects had hypertension, hypercholesterolaemia or glucose intolerance according to the following criteria: (1) resting systolic and diastolic blood pressure exceeding 150 mmHg and 90 mmHg, respectively; (2) total fasting serum cholesterol levels greater than 250 mg. dl⁻¹; (3) a history of insulin-dependent diabetes mellitus or elevated fasting blood sugar levels in excess of 100 mg. dl⁻¹.

Fifty of the 55 patients with valvular heart disease were treated orally with digoxin (0.125–0.25 mg. day⁻¹). Diuretics, including furosemide (20–80 mg. day⁻¹) and/or spironolactone (25–50 mg. day⁻¹) were administered orally to 45 of these patients for at least one month. Diuretic treatment was usually stopped on the morning of the study to avoid frequent urination during the study. A few of the valvular heart disease patients were also treated with nitroglycerin (n=4) or angiotensin converting enzyme inhibitors (n=4) for vasodilation. These vasodilatory drugs were discontinued at least 48 h before the forearm study. In 13 patients with mitral stenotic lesions, a small dose of warfarin (1–3 mg. day⁻¹) was administered to prevent thromboembolic events. In terms of the clinical severity of chronic heart failure, 10 patients were in New York Heart Association functional class I, 26 were in functional class II and 19 were in functional class III (Table 1).

To compare the vasodilatory reaction among the different aetiologies of heart failure, 13 patients with congenital heart disease (mean age = 53±4) were also recruited (Table 1). None of these patients showed evidence of hypertension, hypercholesterolaemia or hyperglycaemia defined by the criteria above. Furosemide was administered orally to these patients with or without digoxin. The number of patients with heart failure due to congenital heart disease were three in functional class I, six in function class II and four in functional class III (Table 1).

None of these patients had gross peripheral oedema or clinical evidence of peripheral vascular disorders and all were clinically stable at the time of the study. Alcohol, caffeine and smoking were prohibited for 6 h period preceding the study. Measurements of capillary wedge pressure (n=57) and cardiac index (n=41) were performed within one month before or after the forearm study.

Forearm plethysmography

Forearm blood flow was measured using plethysmography. After forearm volume had been measured by water displacement, a disposable 20-G arterial cathether with a guiding wire (PA-04020, Arrow International, Reading, PA, U.S.A.) was inserted into the brachial artery in the non-dominant arm under local anaesthesia (1% procainamide). A venous occlusion cuff was attached to the upper arm and a paediatric cuff to the wrist. A gallium-indium-in-silicone rubber strain gauge (model SG-24,
Medasonic, Mountain View, CA, U.S.A.) was carefully placed on the upper third of the forearm, resting comfortably on a support slightly above the level of the heart. To measure forearm blood flow, the wrist cuff was inflated to 160–180 mmHg; the upper arm cuff was then inflated to 40 mmHg using a rapid cuff inflator (model E20, Hokanson, Bellevue, W, U.S.A.) for 7-5 s in each 15-s cycle. After the initiation of strain gauge plethysmography, a minimum period of 20 min was allowed for the subjects to become accustomed to the experimental conditions before blood flow measurement was started. The mean value of the data from the final minute of the recording interval for each dose of each agent was analysed by a computer-assisted digital board, and the results used for statistical analysis.

The protocol consisted of experimental infusions of acetylcholine and sodium nitroprusside. The order of the two agents was randomized, and a minimum recovery period of 20 min was interposed between infusions to allow blood flow to return to baseline levels. Once baseline recordings had been completed for at least 1 min, acetylcholine (at a rate of 0·75, 1·5, 3·0 and 4·5 µg . min⁻¹, 100 ml tissue⁻¹) or sodium nitroprusside (at a rate of 0·05, 0·1, 0·2 and 0·3 µg . min⁻¹, 100 ml tissue⁻¹) was infused through the arterial cannula. These agents were diluted in heparinized physiological saline according to a previously prepared forearm volume/dilution curve. Each dose of experimental agent was infused for 2 min to a maximum infusion volume of 0·5 ml . min⁻¹.

This experimental protocol was approved by our hospital ethics committee, and informed consent was obtained from all subjects.

Statistical analysis
Data are presented as mean ± SE. Differences in baseline characteristics among the groups were tested using the unpaired test, the chi-square test, or one-way ANOVA followed by Fisher's test of least significance. The difference in forearm blood flow from baseline values between the two groups was analysed by two-way ANOVA for repeated measurements followed by Fisher's test of least significance. To analyse the relationships or differences in forearm blood flow changes among the subject group (i.e. as categorized by aetiology and severity), the responses for acetylcholine and nitroprusside were cumulated over the four respective doses compared to the baseline and termed 'acetylcholine response' and 'nitroprusside response' respectively. All calculated P values are two-tailed, and a value of <0·05 was considered to be significant.

Results

Valvular heart disease
The age and sex of patients with valvular heart disease did not differ significantly from healthy controls (Table 1). Systolic blood pressure and heart rate were similar to those in the control group, while diastolic blood pressure was lower in the patient group (71 ± 2 vs 60 ± 2 mmHg, P<0·01; Table 1). However, when patients with dominant aortic regurgitation (n=18) were excluded, the mean diastolic pressure was found not to differ significantly between the two groups (71 ± 2 vs 66 ± 2 mmHg, ns). The mean values for fasting blood sugar levels and total serum cholesterol levels were within the normal range, and did not differ between controls and patients with valvular heart disease (Table 1).

Smoking status was not significantly different between the two groups (Table 1). Non-smokers, including ex-smokers who have not smoked for at least one year, accounted for 71% of the control group and 83% of the patient group (ns). Baseline forearm blood flow and forearm volume were significantly reduced in patients with valvular heart disease (baseline blood flow, 2·9 ± 0·3 vs 2·1 ± 0·1 ml. min⁻¹, 100 ml⁻¹; forearm volume, 831 ± 32 vs 687 ± 19 ml: both P<0·01) (Table 1).

As demonstrated in Fig. 1, forearm blood flow was significantly lower in the patient group than in the control group when mediated by graded doses of both acetylcholine (F=8·11, P<0·001) and sodium nitroprusside (F=5·58, P<0·001).

Congenital heart disease
Blood flow responses in the congenital heart disease group were also compared to the controls to determine whether the vasodilatory effect of acetylcholine and sodium nitroprusside were also impaired in this group. The mean age of the patient group was similar to that of the control group (56 ± 2 vs 53 ± 4 years ns). Sex, systemic blood pressure, heart rate, fasting blood sugar levels, serum cholesterol levels and smoking status in the patient group were matched with the control group (Table 1).

The vasodilatory effects of both acetylcholine and nitroprusside were also clearly attenuated (acetylcholine; F=9·26, P<0·001: nitroprusside; F=10·4, P<0·001: Fig. 2). When the data for acetylcholine and nitroprusside responses were compared between the two aetiologies of heart failure (congenital heart disease vs valvular heart disease), no significant difference was found between the two types of blood flow response (acetylcholine response =10·6 ± 3·2 vs 11·2 ± 1·3 ml . 100 ml⁻¹, ns: nitroprusside response =8·7 ± 1·3 vs 12·0 ± 1·0 ml . 100 ml⁻¹, ns).

Relationship to clinical characteristics
Acetylcholine and nitroprusside responses were compared to various clinical parameters in all patients with heart failure (n=68). Age and sex were not related to blood flow response. The two blood flow responses showed no significant correlation with cardiac index...
(acetylcholine response, r = 0.11; nitroprusside response, r = 0.18; n = 41) or capillary wedge pressure (acetylcholine response, r = -0.24; nitroprusside response, r = -0.18; n = 57). When the total patient sample was divided into three groups according to the clinical severity of heart failure (New York Heart Association functional class), no significant differences in age, sex, aetiology of heart failure, smoking status, fasting blood sugar levels, total serum cholesterol levels and baseline forearm blood flow were found among the groups (Table 2). As shown in Fig. 3, although the nitroprusside response in the very mild heart failure group (functional class I) did not differ significantly from the control value (15.9 ± 1.4 vs 16.4 ± 2.0 ml. 100 ml⁻¹; ns), a significant difference was found in the acetylcholine response (21.1 ± 2.5 vs 16.0 ± 2.5 ml. 100 ml⁻¹; P < 0.01). In the mild to moderate heart failure groups (functional classes II and III), however, forearm blood flow responses to both agents were significantly depressed relative to the severity of heart failure (Fig. 3).

Discussion

This study has demonstrated that acetylcholine-mediated endothelium-dependent vasodilation and nitroprusside-mediated endothelium-independent vasodilation are both impaired in symptomatic patients with chronic heart failure secondary to valvular heart disease or congenital heart disease. We have also shown that these forms of vascular dysfunction appear to advance with the progression of heart failure.

Previous studies

Several recent studies have reported an impairment of cholinomimetic mediated endothelium-dependent vasorelaxation in patients with heart failure due principally to ischaemic coronary heart disease, although an increase in blood flow mediated by nitrovasodilators is preserved in the peripheral vascular bed. However, a
**Table 2** Comparison of clinical characteristics among patient groups determined by New York Heart Association classification

<table>
<thead>
<tr>
<th>NYHA I (n=13)</th>
<th>NYHA II (n=32)</th>
<th>NYHA III (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>54 ±3</td>
<td>59 ±3</td>
</tr>
<tr>
<td><strong>Female/male</strong></td>
<td>7/6</td>
<td>19/13</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>112 ±3</td>
<td>114 ±4</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>66 ±5</td>
<td>68 ±3</td>
</tr>
<tr>
<td><strong>Heart rate (beats . min⁻¹)</strong></td>
<td>62 ±2</td>
<td>63 ±2</td>
</tr>
<tr>
<td><strong>Fasting blood sugar (mg . dl⁻¹)</strong></td>
<td>90 ±2</td>
<td>87 ±1</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg . dl⁻¹)</strong></td>
<td>176 ±7</td>
<td>184 ±5</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td>non-/ex-/smoker</td>
<td>9/3/1</td>
</tr>
</tbody>
</table>

BP=blood pressure; CHD = congenital heart disease; FBF = forearm blood flow; NYHA = New York Heart Association class; VHD = valvular heart disease.

*P<0.01 vs NYHA I

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A classical study conducted by Zelis *et al.* demonstrated a clear reduction in calf blood flow induced by intra-arterial injection of sodium nitrite in patients with chronic heart failure due to valvular heart disease and cardiomyopathy. Katz *et al.* reported an impairment in blood flow velocity in the femoral artery mediated by intra-arterial infusion of nitroglycerin in patients with chronic heart failure, as shown by transcutaneous Doppler ultrasonography. Creager *et al.* noted that nitroprusside-mediated vasodilation was depressed in patients with severe heart failure. Furthermore, two recent reports have suggested that peak forearm blood flow induced by intra-arterial graded administration of nitroglycerin in patients with dilated cardiomyopathy was less than that in healthy subjects. However, no study has defined whether endothelial function is selectively impaired in the peripheral vascular bed in all types of heart failure.

There may be several possible explanations for the differing results concerning nitrovasodilator-induced peripheral vasorelaxation in patients with chronic heart failure. Previous reports investigating the effects of the two types of vasodilator on peripheral blood flow were based on comparisons between relatively small numbers of subjects, i.e. less than 10 per group. As a consequence, the findings arising from these studies may not have sufficient statistical power to discriminate between patients with heart failure and healthy controls with respect to blood flow response to nitrovasodilators. Furthermore, the present study made use of carefully selected patients with chronic heart failure who had no underlying confounding factors such as hypertension, hypercholesterolaemia, or diabetes mellitus, as endothelium-dependent vasodilation has been reported to be impaired in these disorders. However, in a previous study, one third of patients with heart failure had increased serum cholesterol levels, while another did not mention these complicating factors. And although smoking has been suggested as impairing endothelium-dependent vasodilation, smoking status was matched among the groups in our study. If any study is...
Chronic heart failure may therefore be hypothesized to be associated with various acute and chronic changes in the vasculature. It may be documented for several acutely acting vasoactive agents, but tolerance and receptor down-regulation have been shown in the peripheral vascular beds of patients with chronic heart failure. Several recent studies using natriuretic peptide agents have suggested that the cyclic GMP-dependent intracellular messenger system, a common signal transduction mechanism for nitrovasodilators, is impaired in the peripheral vascular beds of patients with chronic heart failure.

**Possible mechanisms**

In the present study, patients with very mild heart failure showed a selective decrease in acetylcholine-mediated flow. In a rat model of heart failure, aortic endothelial dysfunction has been reported to progress over time with the duration of heart failure. In end-stage chronic heart failure, both types of vasodilatation appear to be impaired. We have recently shown in the forearm vascular bed that endothelial-dependent vasodilatation mediated by acetylcholine infusion was rapidly restored after recovery from heart failure due to valve replacement or repair, whereas nitroprusside-mediated vasodilation was not restored. These findings suggest that the severity and duration of heart failure are important factors in vascular dysfunction. The exact duration of symptoms of heart failure could not be defined in our patients, because their physical activity was usually adapted to slow progression of the disease. It may be supposed, however, that duration of heart failure in the present study differed from that observed in previous reports. Symptoms of heart failure due to valvular or congenital heart disease may present earlier than those due to ischaemic heart disease, where symptoms of heart failure usually appear and develop after middle age.

Endothelial function may diminish in the early phase of heart failure, while vascular smooth muscle structure or function may alter over time, possibly due to chronic neurohormonal activation (i.e. angiotensin II, catecholamine) and endothelial paracrine dysregulation of vasoactive substances and cytokines (i.e. endothelin, interleukins, tumour necrosis factor). Chronic activation of these factors may modulate not only vascular smooth muscle cell growth but also extracellular matrix growth, including collagen fibrils and elastic fibres.

In fact, the thickness of capillary basement membrane is increased in forearm muscular tissue in patients with rheumatic valvular heart disease and severe heart failure. Arteriolar hyalinosis in skin biopsy tissue has been demonstrated in patients with idiopathic dilated cardiomyopathy. Similarly, Giannattasio et al. have recently reported that arterial compliance and arterial compliance modulation are impaired even in patients with mild congestive heart failure. Furthermore, tolerance and receptor down-regulation have been documented for several acute and chronic acting vasoactive agents. Several recent studies using natriuretic peptides have suggested that the cyclic GMP-dependent intracellular messenger system, a common signal transduction mechanism for nitrovasodilators, is impaired in the peripheral vascular beds of patients with chronic heart failure. It may therefore be hypothesized that these structural and functional changes in the vessel wall may limit the vasorelaxing effects of nitrovasodilators.

**Clinical implications**

Impaired vasoactivity and capacity is one of the mechanisms that reduces exercise tolerance in patients with congestive heart failure. Peak peripheral blood flow induced by limb occlusion may be correlated with peak oxygen uptake in patients with heart failure. These observations indicate that reduced peripheral blood flow and impaired vasoactivity function may result in an insufficient rise in nutritive blood flow in the exercising skeletal muscles, partly contributing to exercise intolerance. Moreover, since a chronic decrease in blood flow reduces arterial diameter, long-term sedentary conditions after heart failure may reduce vessel size, and then further decrease peripheral circulation and raise cardiac afterload.

**Study limitations**

Basal blood flow in the patient group was lower than that in the control group. Because arterial blood concentrations of nitroprusside were not measured, there may be less dilution of injected nitroprusside due to decreased forearm blood flow in the patient group. In view of this, the observed vasoactivity capacity in patients with chronic heart failure may have been overestimated. This limitation, however, would not have invalidated our conclusions. It may be hypothesized that sodium and water retention in the vessel wall induced myogenic stiffness and reduced vasoactivity. However, none of the patients enrolled in the study showed peripheral oedema. Sinoway et al. showed a rapid increase in metabolic vasoactivity after forearm occlusion following diuretic therapy for heart failure. As diuretics were administered for at least one month before the study, interstitial oedema in the vessel wall would be an unlikely explanation for our results. Although long acting nitrergic was stopped 48 h before commencement of the study, some residual pharmacological effects and nitrate intolerance among the patient groups cannot be entirely ruled out. However, only four of the 55 patients with valvular heart disease and none of those with congenital heart disease were receiving the drug, so there is little possibility of any significant effect on our results.

**Conclusions**

The response of peripheral vasoactivity to endothelium-dependent and endothelium-independent mechanisms is significantly impaired with the progression of heart failure due to non-ischaemic heart disease, where no confounding factors impacting on endothelial function are present.
References


