Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm

Serial assessment in patients with congestive heart failure

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Background In patients with heart failure endothelium-dependent vasodilation of the forearm conduit vessels is impaired possibly because of elevated plasma levels of pro-inflammatory cytokines. The effect of elevated plasma cytokines on endothelium-dependent vasodilation of forearm conduit vessels was therefore serially investigated in 16 patients with congestive heart failure during an episode of acute failure and at the time of recompensation.

Methods and Results Pro-inflammatory cytokine levels and hyperaemic brachial artery diameters were obtained shortly after admission for an episode of acute heart failure and 11±3 days later at the time of recompensation, which was obtained using diuretic therapy without changing other cardiovascular medications. Serum concentrations (Mean±SD) of tumour necrosis factor alpha (TNF-α) (decompensation vs recompensation: 25±23 pg ml⁻¹ vs 26±17 pg ml⁻¹) and interleukine 6 (IL-6) (decompensation vs recompensation: 27±24 pg ml⁻¹ vs 20±18 pg ml⁻¹), determined in venous blood using immunoradiometric assays were elevated but remained unaltered following recompensation. Brachial artery diameter, derived from high-resolution ultrasound scans at rest and during reactive hyperaemia, 90 s after forearm cuff deflation, increased significantly during reactive hyperaemia at the time of admission (3·4±0·7 mm vs 4·0±0·5 mm; P=0·014) and following recompensation (3·4±0·5 mm vs 3·8±0·2 mm; P=0·032). The brachial artery diameter during recompensation expressed as a percentage of the baseline value was similar at both intervals (decompensation vs recompensation: 117±14% vs 116±10%; P=ns). At the time of decompensation, the correlation between TNF-α and the percentage change in brachial artery diameter following reactive hyperaemia was absent (r=0·098; P=0·719). The same correlation became significant at the time of recompensation (r=0·750; P=0·001).

Conclusions In patients with congestive heart failure, plasma levels of pro-inflammatory cytokines correlate with endothelium-dependent vasodilation of the brachial artery following recompensation, but not during an acute episode of heart failure.

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Key Words: Endothelium-dependent vasodilation, heart failure, pro-inflammatory cytokines.

Introduction

Elevated plasma concentrations of pro-inflammatory cytokines such as Interleukine-1α, Interleukine-1β andTNF-α have been reported in patients with heart failure, irrespective of its underlying cause[1,2]. Apart from local action on immune activation and coagulation[3], elevated levels of pro-inflammatory cytokines play a pivotal role in the pathophysiology of congestive heart failure by altering vasomotoricity[4], by causing skeletal muscle wasting[5,6], by reducing myocardial contractility[7] and as recently demonstrated in transgenic mice by inducing myocardial hypertrophy[8]. The vasomotor response is altered by TNF-α through various mechanisms: decreased expression of the constitutive form of nitric oxide synthase (cNOS) together with increased expression of the inducible form of nitric oxide synthase (iNOS) in vascular endothelial cells and vascular smooth cells[9], enhanced production of superoxide anion[10] and raised intracellular levels of tetrahydrobiopterin[11,12].

Through interference with endothelial nitric oxide generation, elevated pro-inflammatory cytokines could account for the impaired endothelium-dependent vasodilation of conduit arteries in response to physiological stimuli in patients with heart failure. Previous studies have described a direct relationship between increased TNF-α levels and nitric oxide mediated
endothelium-dependent vasodilation of the brachial artery in heart failure\cite{4}.

The present study was set up to investigate time-dependent changes in this relationship in patients with congestive heart failure by obtaining serum TNF-α, IL-6 levels and brachial artery diameters at rest and following reactive hyperaemia shortly after admission for an episode of decompensation and following recompensation.

Methods

Study population

The study group consisted of 16 patients, 13 males and three females, with a mean age of 64 ± 12 years (range 42–74 years) admitted with an acute episode of congestive heart failure. All patients had depressed left ventricular function with an ejection fraction below 35% on left ventricular angiography, at the time of diagnostic heart catheterization. Diagnosis of ischaemic heart disease was made in nine patients, idiopathic dilated cardiomyopathy was present in seven patients. No patient had clinical or laboratory evidence of diabetes mellitus. Patients with atrial fibrillation were excluded from the study.

Data were obtained immediately after admission and 11 ± 3 days later following recompensation. Therapy for congestive heart failure consisted of angiotensin-converting enzyme inhibitors in nine, furosemide in 14 and digoxin in six patients. No patient received long acting nitrates, calcium channel blockers or beta adrenergic antagonists during the study period. Therapy for recompensation consisted of loop diuretics in 16 patients and of potassium sparing diuretics, administered intravenously and/or orally in four patients. Non-diuretic therapy remained unaltered during the study.

Cytokine measurements

Plasma levels of IL-6 and TNF-α were measured on the day of admission and at recompensation. Blood samples were centrifuged at 3000 g for 15 min and plasma was stored at −20 °C.

TNF-α and IL-6 were measured by immunoradiometric assay (Medgenix/Diagnostics SA; Fleurus Belgium) based on coated-tube separation and on the oligoclonal system, in which several monoclonal antibodies, directed against distinct epitopes of, respectively, TNF-α and IL-6 are used. These assays have a high specificity and the lower limit of detection was 5 pg . ml\(^{-1}\) for TNF-α and 6 pg . ml\(^{-1}\) for IL-6\cite{13}. If necessary, samples with high levels of IL-6 or TNF-α were diluted and reassayed. All samples were run in duplicate; the average of two measurements is reported.

Forearm vessel diameter measurements

Brachial artery diameters were serially measured on two-dimensional ultrasound images using a 7 MHz linear array transducer and a standard 125 XP/10 system (Acuson, CA, U.S.A.). This 7-0 MHz ultrasound system has a theoretical limit of axial resolution in the near field of about 0-1 to 0-2 mm.

For accurate repetitive measurements of the brachial artery, anatomical markers such as bifurcations were used. Longitudinal and transverse scans from the brachial artery were made and diameter measurements were taken from the anterior to the posterior M-mode line at end-systole\cite{14}. All patients rested comfortably for at least 10 min with the head slightly elevated above heart level. Each study consisted of resting scan recordings, forearm cuff inflation for 3 min and a second scan 90 s following cuff deflation. Vessel diameters from scans during reactive hyperaemia were expressed as a percentage of the resting scan value.

Data analysis

All results are shown as mean ± standard deviation. Single comparison data were analysed using a paired or unpaired Student’s t-test as appropriate. Forearm changes in diameter and cytokines were correlated with linear regression (least-squares method). Statistical significance was set at a two-tailed probability level of less than 0-05.

Results

Haemodynamic parameters and serial plasma cytokine measurements

Data are summarized in Table 1 and Fig. 1. Recompensation resulted in a significant loss of weight (decompensation vs recompensation: 94 ± 10 kg vs 88 ± 12 kg; \(P=0.001\)) together with a reduction in heart rate from 118 ± 20 beats . min\(^{-1}\) to 92 ± 10 beats . min\(^{-1}\) (\(P=0.04\)). The mean arterial blood pressure remained unchanged (decompensation vs recompensation: 82 ± 18 mmHg vs 87 ± 20 mmHg; \(P=ns\)).

Serum concentrations of TNF-α were above the detection limit in all patients, with a mean serum concentration of 25 ± 23 pg . ml\(^{-1}\) (range 12 to 110 pg . ml\(^{-1}\)) at time of admission and a mean serum concentration of 26 ± 17 pg . ml\(^{-1}\) (range 15 to 86 pg . ml\(^{-1}\)) following recompensation. These values are significantly higher than values observed in a control population of healthy subjects (8 ± 7 ± 4·9 pg . ml\(^{-1}\))\cite{22}. Recompensation did not result in statistically significant changes in TNF-α (25 ± 23 pg . ml\(^{-1}\) vs 26 ± 17 pg . ml\(^{-1}\); \(P=0.89\)). Similar concentrations of TNF-α were found at both intervals (Fig. 1) (decompensation:
The levels of IL-6 measured at the acute episode were above the detection limit in 15 patients (93%) with a mean value of 27 ± 24 pg.mL⁻¹ (range 6 to 95 pg.mL⁻¹) and in 12 patients (75%) following recompensation, with a mean value of 20 ± 18 pg.mL⁻¹ (range 6 to 57 pg.mL⁻¹). Although recompensation did not result in significantly lower IL-6 levels (27 ± 24 pg.mL⁻¹ vs 20 ± 18 pg.mL⁻¹, \( P=0.08 \)), there was a trend towards fewer patients with detectable IL-6 levels at follow-up. Compared to patients with ischaemic cardiomyopathy, those with non-ischaemic dilated cardiomyopathy had similar IL-6 levels at the time of decompensation (33 ± 27 pg.mL⁻¹ vs 19 ± 17 pg.mL⁻¹, \( P=\text{ns} \)) and at the time of recompensation (23 ± 20 pg.mL⁻¹ vs 16 ± 15 pg.mL⁻¹, \( P=\text{ns} \)).

The effects of recompensation upon haemodynamic parameters, TNF-α, IL-6, brachial artery diameter at rest and following reactive hyperaemia are shown in Table 1.

### Table 1: Effect of recompensation upon haemodynamic parameters, TNF-α, IL-6, brachial artery diameter at rest and following reactive hyperaemia

<table>
<thead>
<tr>
<th></th>
<th>Decompensation</th>
<th>Recompensation</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>94 ± 10</td>
<td>88 ± 12</td>
<td>0.001</td>
</tr>
<tr>
<td>HR (beats.min⁻¹)</td>
<td>118 ± 20</td>
<td>92 ± 10</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>82 ± 18</td>
<td>87 ± 20</td>
<td>ns</td>
</tr>
<tr>
<td>TNF-α (pg.mL⁻¹)</td>
<td>25 ± 23</td>
<td>26 ± 17</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6 (pg.mL⁻¹)</td>
<td>27 ± 24</td>
<td>20 ± 18</td>
<td>ns</td>
</tr>
<tr>
<td>BAD (mm)</td>
<td>3.4 ± 0.7</td>
<td>3.4 ± 0.5</td>
<td>ns</td>
</tr>
<tr>
<td>ED-BAD (mm)</td>
<td>4.0 ± 0.5*</td>
<td>3.8 ± 0.2**</td>
<td>ns</td>
</tr>
<tr>
<td>% BAD</td>
<td>117 ± 14</td>
<td>112 ± 6</td>
<td>ns</td>
</tr>
</tbody>
</table>

HR = heart rate; BP = blood pressure; BAD = brachial artery diameter; ED-BAD = brachial artery diameter following reactive hyperaemia; % BAD = percent change in brachial artery diameter following reactive hyperaemia. *\( P=0.014 \) compared to BAD; **\( P=0.032 \) compared to BAD.

### Figure 1: Scatterplot showing individual concentrations of TNF-α and IL-6 at the time of decompensation (decomp) and following recompensation (recomp). Individual values are expressed as open circles whereas the closed circles represent the mean concentration ± SD.

### Figure 2: Bar graph showing mean brachial artery diameter ± SD at baseline (□) and following reactive hyperaemia (◼) at the time of decompensation and following recompensation. Hyperaemia resulted in a significant increase in brachial artery diameter. *\( P=0.014 \); **\( P=0.032 \).
Brachial artery diameters at rest and during reactive hyperaemia

Data are summarized in Table 1 and Fig. 2. In all subjects, scans were of sufficient quality for assessment of vessel diameter and response. All brachial arteries were free of atherosclerotic plaques. Recompensation using diuretic therapy did not result in statistically significant changes in baseline brachial artery diameter (decompensation vs recompensation: 3.4±0.7 mm vs 3.4±0.5 mm; P=0.09). After 3 min of arterial occlusion, reactive hyperaemia resulted in a significant increase in brachial artery diameter (acute episode: 3.4±0.7 mm vs 4.0±0.5 mm; P=0.014; recompensation: 3.4±0.5 mm vs 3.8±0.2 mm; P=0.032) (Fig. 2). The percentage dilation during reactive hyperaemia was similar at the time of both decompensation and recompensation (117±14% vs 112±6%; P=0.152).

Correlation between plasma cytokines and brachial artery diameter

Flow mediated brachial artery vasodilation was closely correlated with TNF-α levels at the time of recompensation (P=0.001, r=0.750) (Fig. 3). The same correlation was absent at the time of admission for an episode of acute decompensation (P=0.719, r=0.098) (Fig. 4).

Discussion

In the present study we demonstrated increased levels of TNF-α in all, and increased levels of IL-6 in 93% of patients with congestive heart failure at the time of admission with an episode of acute failure. TNF-α and IL-6 plasma levels did not appear to depend on...
underlying cardiac disease. Following recompensation, TNF-α concentrations remained unaltered while there was a trend for fewer detectable IL-6 levels. Nitric oxide mediated endothelium-dependent vasodilation of the conduit arteries remained unaltered following recompensation. Resolution of the acute episode of congestive heart failure following therapy with diuretics resulted in restoration of the relationship between TNF-α and flow mediated endothelium-dependent vasodilation of the brachial artery.

**Pro-inflammatory cytokines**

In a variety of cardiac diseases which are not attributable to bacterial infections, like viral myocarditis and myocardial infarction, increased levels of pro-inflammatory cytokines are observed. Although the pro-inflammatory cytokine levels in our study were lower compared to those observed during sepsis and endotoxaemia, they appear elevated compared to normal healthy subjects and are similar to those previously reported in patients with NYHA II and III heart failure. At the time of admission for an episode of acute failure we found detectable concentrations of TNF-α in all patients while IL-6 concentrations were above the detection limit in 93% of patients. The trend for lower IL-6 levels following recompensation suggests that, in contrast to TNF-α, IL-6 behaves like an acute phase reactant. Exacerbation results in a rapid surge of IL-6 in patients with congestive heart failure. A similar time course of plasma IL-6 level has been observed following myocardial infarction where IL-6 level increases quickly, with a peak between day 3 and 7. In vitro experiments demonstrate a rise in myocardial IL-6 production when cardiac myocytes are subjected to hypoxic conditions, the trend for higher IL-6 levels at the time of admission could result from myocardial oxygen deprivation during the episode of acute heart failure.

**Pro-inflammatory cytokines and endothelium-dependent vasodilation**

The impaired ability of blood vessels to dilate in response to physiological stimuli contributes to the reduced exercise tolerance characteristic of congestive heart failure patients. Part of this reduced vasodilator capacity is due to activation of the orthosympathetic and renin-angiotensin systems. Other mechanisms responsible for this blunted vasodilation are the attenuated release of nitric oxide from the vascular endothelium and the enhanced production of oxygen free radicals. Cytokines, especially TNF-α, interfere with endothelial function by generating oxygen free radicals and modulating endothelial NO release. In vitro experiments suggest that TNF-α enhances superoxide radical generation by induction of a p22 phox containing NADH oxidase. This oxidative stress causes apoptosis of the endothelium and inactivation of nitric oxide generated by the endothelium.

Through expression of inducible nitric oxide synthase, cytokines modulate vasomotor tone and precipitate peripheral vasodilation as seen in septic shock. TNF-α is known to induce the expression of an inducible form of nitric oxide synthase (iNOS) in endothelial cells and vascular smooth muscle cells and to decrease the expression of the constitutive form of nitric oxide synthase (cNOS) in vascular endothelial cells. In contrast to Ca2+ dependent cNOS which is tightly regulated and releases small amounts of nitric oxide to very specific stimuli, Ca2+ independent iNOS releases large amounts of nitric oxide. In the present study we were able to demonstrate a positive correlation between flow-mediated endothelium-dependent vasodilation and circulating levels of TNF-α following recompensation. Other investigators have also demonstrated a similar correlation between increased TNF-α and forearm blood flow responses to acetylcholine and nitroglycerin in patients with heart failure. By binding directly to the TNF-α molecule or by preventing its binding to cells, these so-called soluble TNF-α receptors (sTNF-R) modulate its bio-activity. In the present study we failed to measure sTNF-R, but as all patients were in NYHA class IV at the time of admission, the concentration of sTNF-Rs must have been higher than following recompensation when patients were in NYHA class II-III. During an episode of acute heart failure, the concentrations of oxygen free radicals are also elevated causing more profound inactivation of endothelial cells.

**Conclusions**

Although the exact role of pro-inflammatory cytokines in the pathogenesis of congestive heart failure remains
speculative and not clearly understood, the present study demonstrates that the interaction between TNF-α and hyperaemia mediated vasodilation of the brachial artery is abolished during an acute episode of congestive heart failure, but restored following recompensation. Enhanced production of oxygen free radicals and decreased bio-activity of TNF-α due to binding with shed TNF-α receptors might be responsible for this observation.

References


