Elevated circulating cardiotrophin-1 in heart failure: relationship with parameters of left ventricular systolic dysfunction

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ABSTRACT
Cardiotrophin-1 (CT-1) is a cytokine that has been implicated as a factor involved in myocardial remodelling. The objective of the present study was to establish the relationship between circulating levels of CT-1 and measures of left ventricular size and systolic function in patients with heart failure. We recruited 15 normal subjects [six male; median age 60 years (range 30–79 years)] and 15 patients [11 male; median age 66 years (range 43–84 years)] with a clinical diagnosis of heart failure and echocardiographic left ventricular systolic dysfunction (LVSD). Echocardiographic variables (left ventricular wall motion index, end-diastolic and -systolic volumes, stroke volume, fractional shortening) and plasma CT-1 levels were determined. In patients with LVSD [median wall motion index 0.6 (range 0.3–1.4)], CT-1 was elevated [median 110.4 fmol/ml (range 33–516 fmol/ml)] compared with controls [wall motion index 2 in all cases; median CT-1 level 34.2 fmol/ml (range 6.9–54.1 fmol/ml); \(P < 0.0001\)]. Log CT-1 was correlated with log wall motion index \((r = -0.76, P < 0.0001)\), log left ventricular end-systolic volume \((r = 0.54, P < 0.05)\), stroke volume \((r = -0.60, P = 0.007)\) and log fractional shortening \((r = -0.70, P = 0.001)\). In a multivariate model of the predictors of log wall motion index, the only significant predictor was log CT-1 \((\chi^2 = 56\%, P = 0.006)\). This is the first assessment of the relationship between plasma CT-1 levels and the degree of LVSD in humans, and demonstrates that CT-1 is elevated in heart failure in relation to the severity of LVSD.

INTRODUCTION
The recently cloned novel cytokine cardiotrophin-1 (CT-1) is a member of the family of interleukin-6-related cytokines that bind to glycoprotein 130 (gp130) receptors. The family includes interleukin-6, interleukin-11, ciliary neurotrophic factor, leukaemia inhibitory factor (LIF) and oncostatin M [1]. CT-1 is expressed at high levels in the myocardium during cardiogenesis, promoting the proliferation and survival of embryonic cardiomyocytes [1–3]. CT-1 induces cardiac myocyte hypertrophy [1], adding sarcomeres in series rather than in parallel and leading to increased cardiac myocyte size due to an increase in cell length, with little change in width [2]. CT-1 binds to the LIF receptor, leading to tyrosine phosphorylation of the gp130/LIF receptor heterodimer [2,3]. Signalling cascades involved include the Janus kinase (JAK)/signal

Key words: cardiotrophin-1, cytokines, echocardiography, heart failure.
Abbreviations: ACE, angiotensin-converting enzyme; BNP, brain (B-type) natriuretic peptide; CT-1, cardiotrophin-1; EDV, left ventricular end-diastolic volume; ESV, left ventricular end-systolic volume; FS, fractional shortening; gp130, glycoprotein 130; JAK, Janus kinase; LIF, leukaemia inhibitory factor; LVID, left ventricular internal dimensions; LVIDd, LVID at end-diastole; LVIDs, LVID at end-systole; LVSD, left ventricular systolic dysfunction; STAT, signal transducer and activation of transcription; SV, stroke volume; WMI, wall motion index.
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transducer and activation of transcription 3 (STAT3) pathway [4].

In addition to its effects on cardiomyocyte hypertrophy, CT-1 has also been shown to prevent myocyte apoptosis via a pathway dependent on activation of a mitogen-activated protein kinase [5]. Further evidence of a cardiac cytoprotective effect was evident in experiments where CT-1 was shown to induce accumulation of heat-shock protein in cultured cardiac cells, protecting them from ischaemic stress [6]. The hypertrophic response of cardiac myocytes to CT-1 is dependent on the JAK/STAT pathway, whereas the anti-apoptotic effects of CT-1 employ signalling via the mitogen-activated protein kinase pathway.

In experimental animals, the acute haemodynamic effects of CT-1 include an increase in cardiac output via an increase in heart rate, and a decrease in systemic vascular resistance without a direct positive inotropic effect [7]. In addition, CT-1 stimulates the production of brain (B-type) natriuretic peptide (BNP) at a transcriptional level by a mechanism that is distinct from the effect of endothelin-1 on cultured neonatal rat cardiomyocytes [8,9].

Increased expression of CT-1 occurs in a rat model of acute myocardial infarction, and this may relate to a role for CT-1 in the pathogenesis of ventricular remodelling [10]. In addition, overexpression of cardiac CT-1 and gp130 occurs during experimental heart failure [11]. Cardiomyocytes from humans with ischaemic cardiomyopathy are about 50% longer than normal, suggesting the addition of sarcomeres in series as WMI [11,12]. As noted above, CT-1 induces cardiac myocyte hypertrophy via the addition of sarcomeres in series rather than in parallel and via increased cardiac myocyte length with little change in width. These data suggest a possible role for CT-1 in ventricular remodelling following acute myocardial infarction in humans, and also in patients with established heart failure. There are currently no data on circulating CT-1 levels in subjects with LVSD.

We hypothesized that CT-1 levels would be raised in subjects with LVSD. We thus examined whether CT-1 is detectable in the plasma of normal humans, whether plasma levels are elevated in patients with heart failure, and the relationship between plasma CT-1 levels and measures of LVSD. We used a competitive immunoluminometric assay for detecting circulating CT-1 using techniques developed for the measurement of plasma levels of N-terminal proBNP [13].

METHODS

Subjects
We studied 15 consecutive patients with a clinical diagnosis of heart failure and echocardiographic LVSD [11 male; median age 66 years (range 43–84 years)] and 15 normal subjects [six male; median age 60 years (range 30–79 years)]. Normal subjects were nine individuals [four male; median age 40 years (range 30–79 years)] with normal echocardiographic examination and six healthy volunteers [two male; median age 63 years (range 48–79 years)]. None of the normal subjects was taking any prescribed medication and none had any history of cardiovascular disease. Of the patients with LVSD, 10 were receiving a loop diuretic, seven an angiotensin-converting enzyme (ACE) inhibitor, two digoxin and one a β-blocker.

Patients and subjects with normal echocardiographic examination were recruited during attendance for a routine outpatient echocardiographic examination. Healthy volunteers were the spouses or partners of patients. The study was approved by the Leicester Health Authority Ethics Committee. All subjects gave written informed consent to participation.

Echocardiography
Echocardiography was performed using a Hewlett Packard Sonos 1500 imaging system. Wall motion index (WMI) was calculated using a validated nine-segment model [14,15], blind to patient details. LVSD was defined as WMI ≤ 1.4, corresponding to a left ventricular ejection fraction of ≤ 35%. Left ventricular chamber dimensions were obtained at the tip of the mitral valve. Interventricular septal thickness and posterior wall thickness were measured at end-diastole. Left ventricular internal dimensions (LVID) were obtained at end-diastole (LVIDd) and at end-systole (LVIDs). Fractional shortening (FS) was calculated as the percentage change in the LVID between systole and diastole. Left ventricular volumes were calculated according to the formula of Teicholz et al. [16]. Left ventricular stroke volume (SV) was defined as the left ventricular end-diastolic volume (EDV) minus the left ventricular end-systolic volume (ESV).

Blood sampling
Blood sampling was carried out following echocardiographic examination. Following 30–45 min of supine rest, 20 ml of blood was transferred to pre-chilled tubes containing 500 units/ml aprotinin (Trasylol; Bayer UK, Newbury, U.K.) and EDTA (1.5 mg/ml). Plasma was separated immediately and stored at –70 °C until assay for CT-1 within 2 months.

Assay for CT-1
Details of the immunoluminometric competitive binding assay for CT-1 have been described in full previously [17]. Briefly, stored plasma samples were extracted using C$_{18}$ extraction cartridges and redissolved in assay buffer for measurement of CT-1. The CT-1 peptide was
synthesized in the Medical Research Council Toxicology Unit, Leicester University, and conjugated to keyhole-limpet haemocyanin to immunize rabbits by monthly subcutaneous injections. An in-house polyclonal antibody to amino acids 105–120 of the CT-1 sequence was developed and a methyl acridinium ester was used to label the peptide. Plasma extracts were incubated with the antibody before addition of the tracer. Immune complexes were recovered with paramagnetic particle immobilized goat anti-rabbit globulin and, after extensive washes, chemiluminescence was measured by sequential injections of acidified H$_2$O$_2$ (0.1 M HNO$_3$) and 0.25 M NaOH. Peptide recovery using the C$_{18}$ column plasma extraction process was 76.2 ± 3%.

Assays were performed using a Berthold Autolumat LB953 luminometer. Within- and between-assay coefficients of variation were 6.2% and 10.3% respectively. Cross-reactivity with other peptides (i.e. atrial natriuretic peptide, BNP, N-terminal proBNP, interleukin-6 and LIF) known to be elevated in heart failure was <0.1% in all cases. CT-1 levels were determined blind to patient details and echocardiographic findings. Each CT-1 value represents the mean of duplicate measurements.

### Statistical analyses

All statistical analyses were carried out using the software package Minitab (Minitab Inc.). All results are expressed as median (range). Values of CT-1, WMI, interventricular septal thickness, posterior wall thickness, FS, ESV, EDV and SV were normalized by logarithmic transformation prior to analyses. For the categorical variables clinical heart failure, current diuretic use and current ACE inhibitor use, 95% confidence intervals were calculated for the difference in plasma CT-1 values between patients with and without the variable of interest. Comparisons between patients with LVSD and control subjects were made using the Mann–Whitney U-test for non-parametric data.

### RESULTS

Echocardiographic data obtained from LVSD subjects and controls are reported in Table 1. Five patients with LVSD were categorized within New York Heart Association class II, eight patients within class III and two patients within class IV. Median WMI in the 15 patients with LVSD was 0.6 (range 0.3–1.4). Plasma CT-1 levels were elevated in patients with LVSD (median 110.4, range 33–516 fmol/ml) compared with control subjects (median 34.2, range 6.9–54.1 fmol/ml; P < 0.0001) (Figure 1).

Positive correlations were apparent between log plasma CT-1 level and a number of echocardiographic indices: log WMI (r = −0.76, P < 0.0001) (Figure 2), log

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with LVSD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMI</td>
<td>0.6 (0.3–1.4)</td>
<td>2***</td>
</tr>
<tr>
<td>FS (%)</td>
<td>17.2 (11.4–25)</td>
<td>46.9 (30.2–59)***</td>
</tr>
<tr>
<td>IVST (cm)</td>
<td>1.15 (0.91–2.04)</td>
<td>0.9 (0.6–1.02)*</td>
</tr>
<tr>
<td>PWT (cm)</td>
<td>1.12 (0.91–2.04)</td>
<td>0.9 (0.58–1.02)*</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>5.03 (3.44–7.41)</td>
<td>5.12 (4.3–5.82)</td>
</tr>
<tr>
<td>LVIDs (cm)</td>
<td>3.93 (2.84–6.16)</td>
<td>2.8 (1.98–3.0)**</td>
</tr>
<tr>
<td>Left atrial diameter (cm)</td>
<td>3.8 (1.92–8.37)</td>
<td>3.5 (2.8–4.47)</td>
</tr>
<tr>
<td>ESV (ml)</td>
<td>67.1 (30.6–191)</td>
<td>29.6 (12.4–35)**</td>
</tr>
<tr>
<td>EDV (ml)</td>
<td>120.5 (48.8–290)</td>
<td>125 (83.1–168)</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>49 (18.2–99)</td>
<td>99.1 (48.1–138)*</td>
</tr>
</tbody>
</table>

† All control subjects had a WMI of 2.

![Figure 1](https://example.com/figure1.png)  
**Figure 1** Plasma CT-1 concentrations in patients with LVSD and in control subjects

Logarithmic transformation has been carried out. Median values are shown as horizontal lines.

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Correlation between log CT-1 and log WMI in patients with LVSD and in control subjects

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Figure 3 Correlation between log CT-1 and log FS in patients with LVSD and in control subjects

Figure 4 Correlation between log CT-1 and log ESV in patients with LVSD and in control subjects

We considered age, gender, history of ischaemic heart disease or hypertension, current diuretic or ACE inhibitor use, presence of clinical left ventricular failure and log CT-1 in a multivariate model of the predictors of log WMI. The only significant predictor was log CT-1 ($P = 0.006$). On best-subset analysis the strongest correlate with log WMI was log CT-1 ($R^2 = 55.5\%$).

We performed further analysis, and looked at determinants of circulating CT-1 levels in our patients with heart failure. In a multiple-regression model the determinants of CT-1 were log WMI ($P < 0.005$), the presence of a history of hypertension ($P = 0.006$), the age of the patient ($P < 0.05$), log interventricular septal thickness ($P = 0.009$), LVIDd ($P < 0.05$), LVIDs ($P < 0.05$), log posterior wall thickness ($P = 0.007$), log ESV ($P < 0.05$) and log EDV ($P < 0.05$). In a best-subset analysis log WMI, history of hypertension, LVIDd and log ESV accounted for 32.6% of the variance in plasma CT-1 levels ($R^2 = 32.6\%$).

**DISCUSSION**

Long-standing exposure to hypertension or haemodynamic stress secondary to ischaemic injury can activate a distinct form of myocardial hypertrophy resulting in cardiac dilatation. This process of remodelling involves the addition of new myocytes in series and results in irreversible loss of myocardial function. The identification of stimuli that initiate and maintain the processes of cardiac hypertrophy and remodelling, and eventually heart failure, remains a major pursuit in cardiac molecular biology and medicine. This is the first report of elevated plasma CT-1 levels in subjects with LVSD. While the exact role of CT-1 in LVSD and its relationship with ventricular remodelling remain to be defined, related cytokines such as tumour necrosis factor-$\alpha$ and certain interleukins are capable of modulating cardiovascular function by a variety of mechanisms, including the promotion of left ventricular remodelling [18,19].

We have demonstrated a strong correlation between the magnitude of the rise in plasma CT-1 levels and the severity of LVSD. Plasma CT-1 levels were particularly correlated with two measures of left ventricular systolic function, namely WMI and FS. Furthermore, we have demonstrated CT-1 level to be a significant predictor of WMI in a multivariate model. Whether measurement of CT-1 levels in patients with known or suspected LVSD can be of clinical use in terms of diagnosis or monitoring of disease progress remains to be tested in larger prospective studies. In this respect, the relative merits of CT-1 in comparison with other neurohormones such as BNP will be of interest.
End-stage heart failure due to ischaemic or dilated cardiomyopathy is characterized by a dilated, thin-walled ventricle. The structural basis for this ventricular dilatation is the side-to-side slippage of myocytes. However, probably of more importance is an increase in the myocyte length secondary to an increase in the numbers of sarcomeres in series [12,20]. The degree of side-to-side slippage of myocytes does not correlate with the magnitude of ventricular enlargement; furthermore, myocyte lengthening alone may account for all the ventricular dilatation seen in cardiomyopathy of various aetiologies [12,20]. Similar changes of myocyte lengthening and maladaptive remodelling of the cardiac myocyte begin in hypertension long before ventricular failure occurs [20].

The mechanism for the initiation and maintenance of increased circulating plasma CT-1 levels in patients with heart failure is unknown. One possibility is that CT-1 expression may be induced by myocyte stretch. Indeed, mechanical stretch activates the JAK/STAT pathway and augments expression of CT-1 mRNA in rat cardiomyocytes [21]. It is intuitive to propose that the initial secretion of CT-1 following ischaemic embarrassment may be adaptive via: (i) cytoprotective effects potentially conferred by this peptide; (ii) improvement in cardiac output and haemodynamics; and (iii) series addition of sarcomeres, resulting in a distribution of augmented wall stress against an increased number of myofibrils. However, prolonged secretion of CT-1 may be deleterious, resulting in excessive ventricular dilatation and irrevocable loss of function.

Our study has a number of important limitations. This early report is limited by the relatively small number of patients studied. We are currently investigating patterns of secretion of CT-1 in larger numbers of patients with chronic heart failure, following acute myocardial infarction and in unstable angina. The tendency to higher levels of CT-1 in patients with clinical heart failure or in those receiving diuretic or ACE inhibitor therapy may simply reflect disease severity. However, the present study does not address the issue of the response of CT-1 to pharmacological treatment of heart failure. Another limitation of the present study is the inability to define a direct cause-and-effect relationship between elevated CT-1 levels and clinical parameters. Further to this, the relationship between CT-1 and other cytokines known to be elevated in heart failure remains to be addressed. In this respect it is of interest to note that CT-1 stimulates BNP gene expression and peptide secretion in cultured rat myocytes [8,9]. This raises the intriguing possibility that CT-1 may represent an earlier phase of the cascade of neuroendocrine activation seen in heart failure. Thus assessment of CT-1 and other neurohormones, such as BNP, may provide differing but complementary information at different stages in the disease process. Clearly additional studies with larger number of patients are required.

The present study has identified a new signalling pathway in human heart failure which could potentially be manipulated therapeutically. Agonists or antagonists of the CT-1 receptor may have therapeutic potential in the protection of cardiac cells from ischaemic injury, particularly if the cytoprotective properties can be separated from the damaging hypertrophy-inducing effects that result from continuous activation of the gp130 signalling pathway. Additional clinical studies are presently in progress, and may shed further light on the role of this peptide in cardiovascular disease.

ACKNOWLEDGMENTS

We thank the British Heart foundation for its support. S.T. was supported by a Leicester Royal Infirmary research fellowship.

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Received 4 January 2000/9 March 2000; accepted 30 March 2000