Increased systemic inflammation and oxidative stress in patients with worsening congestive heart failure: improvement after short-term inotropic support

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ABSTRACT

In the present study, we evaluated circulating pro-inflammatory mediators and markers of oxidative stress in patients with decompensated CHF (congestive heart failure) and assessed whether clinical recompensation by short-term inotropic therapy influences these parameters. Patients with worsening CHF (n = 29, aged 61.9 ± 2.7 years), NYHA (New York Heart Association) class III–IV, and left ventricular ejection fraction of 23.7 ± 1.8% were studied. Controls comprised age-matched healthy volunteers (n = 15; 54.1 ± 3.2 years). Plasma levels of cytokines [IL (interleukin)-6 and IL-18], chemokines [MCP-1 (monocyte chemotactic protein-1)], adhesion molecules [sICAM (soluble intercellular adhesion molecule), sE-selectin (soluble E-selectin)], systemic markers of oxidation [TBARS (thiobarbituric acid-reactive substances), 8-isoprostaglandin F2α, and nitrotyrosine] and hs-CRP (high-sensitivity C-reactive protein) were measured by ELISA and colorimetric assays at admission and 30 days following 72-h milrinone (n = 15) or dobutamine (n = 14) infusion. Plasma IL-6, IL-18, sICAM, E-selectin, hs-CRP and oxidative markers were significantly higher in patients on admission before inotropic treatment compared with controls (P < 0.05). Short-term inotropic support improved clinical status as assessed by NYHA classification and by the 6-min walk test and significantly decreased plasma levels of IL-6, IL-18, sICAM, hs-CRP and markers of oxidation (P < 0.05) at 30 days. The effects of milrinone and dobutamine were similar. In conclusion, our results demonstrate that patients with decompensated CHF have marked systemic inflammation and increased production of oxygen free radicals. Short-term inotropic support improves functional status and reduces indices of inflammation and oxidative stress in patients with decompensated CHF.

Key words: adhesion molecule, cytokine, inflammation, dobutamine, heart failure, milrinone, oxidative stress.

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CHF, congestive heart failure; CRP, C-reactive protein; hs-CRP, high-sensitivity CRP; ICAM, intercellular adhesion molecule; IL, interleukin; LVEF, left ventricular ejection fraction; MCP-1, monocyte chemotactic protein-1; MDA, malondialdehyde; NYHA, New York Heart Association; ROS, reactive oxygen species; sE-selectin, soluble E-selectin; sICAM, soluble ICAM; TBARS, thiobarbituric acid-reactive substances; TNF, tumour necrosis factor.

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INTRODUCTION

CHF (congestive heart failure), characteristically described as a haemodynamic syndrome, is increasingly being recognized as a neuro-endocrine condition [1,2]. Over the recent past, it has become evident that heart failure is associated with subclinical inflammation [1–3], with studies demonstrating non-specific elevation in levels of many pro-inflammatory markers, such as TNF (tumour necrosis factor)-α, IL (interleukin)-1, IL-6, soluble adhesion molecules and CRP (C-reactive protein) [2,4–10]. CHF has also been described as a state of oxidative excess [3]. Generation of ROS (reactive oxygen species) is increased in patients with worsening cardiac failure as evidenced by increased plasma levels of TBARS (thiobarbituric acid-reactive substances), uric acid and pericardial levels of 8-isoprostaglandin F_2α [11,12]. These markers of oxidative stress, which correlate with the functional severity of CHF, may reflect both systemic and local production of free radicals [12–15]. Increased myocardial oxidative stress could lead to activation of redox-sensitive transcription factors and induction of signalling pathways contributing to impaired contraction, endothelial dysfunction, myocyte apoptosis and necrosis, fibroblast proliferation, deposition of extracellular matrix proteins, cardiac remodelling and progressive deterioration of the failing heart [12,14,16,17].

Some clinical investigations have reported changes in inflammatory markers in patients with few symptoms as well as in patients with advanced heart failure [5,18–22]. However, only a few clinical reports have investigated changes in these markers in very sick patients with decompensated CHF requiring inotropic or mechanical support [19,20]. Furthermore, changes in inflammatory markers concomitantly with those of oxidative stress have not been reported in patients with acute worsening CHF. Accordingly, the aim of the present study was to determine whether global markers of inflammation and oxidative stress are altered in patients with decompensated CHF and whether clinical improvement and recompensation mediated by short-term inotropic therapy influences these parameters.

METHODS

Study population
The study complies with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Montreal Heart Institute. All subjects gave informed consent to participate in the study. Patients presenting at the Cardiac Failure Unit of the Montreal Heart Institute between November 1998 and September 2001, with CHF as evidenced by severe fluid overload and/or low cardiac output and who failed to improve with intravenous diuretics, entered into the study. Exclusion criteria included history of inflammatory musculoskeletal disorders, recent infection, fever, severe renal failure (plasma creatinine, > 300 μmol/l) or any other active systemic disease. In addition, patients with heart failure caused by severe anaemia (haemoglobin, < 90 g/l), atrial or ventricular dysrythmias, unstable angina or acute coronary syndrome were excluded from the study.

Eligible patients were randomly assigned to receive either dobutamine or milrinone. Blood samples were collected before the initiation of inotropic support. Milrinone was administered at a mean dose of 0.45 ± 0.34 μg·kg⁻¹·min⁻¹ for 66.3 ± 21.1 h, and dobutamine was infused at a mean dose of 8.9 ± 2.2 μg·kg⁻¹·min⁻¹ for 77.6 ± 34.3 h. Inotropic support was discontinued when patients reached 72 h of infusion or optimal clinical status. Patients returned to the clinic for a second visit and blood was drawn 34.6 ± 8.1 days (range 22–33 days) after randomization. If clinically indicated, a supplementary visit was scheduled between the time of discharge to the second clinic visit. Only patients able to receive at least 24 h of milrinone or dobutamine were included in the study. Fifteen healthy control subjects without apparent cardiovascular conditions and on no medication were recruited. Subjects had blood drawn in the resting state at least 2 h after a light breakfast.

Six-min walk test
The functional status of patients was also evaluated using a 6-min walk test. The test was performed within 24 h of the end of the perfusion of the inotropic agent and at the 30-day visit. The test was performed by the same research nurse or physical therapist at the same time of the day using the method reported by Guyatt et al. [23].

Blood collection
Blood, collected in EDTA tubes, was immediately centrifuged and plasma removed and frozen at −80 °C until assays were performed. To prevent inter-assay variability, samples were stored and all assays were performed at the same time.

Measurement of plasma IL-6, IL-18, MCP-1 (monocyte chemotactic protein-1), sICAM-1 (soluble ICAM (intercellular cell-adhesion molecule)-1), sE-selectin (soluble E-selectin) and nitrotyrosine
All assays were performed by technicians who were unaware of the patient/subject status. Plasma levels of IL-6 and MCP-1 were measured using a solid-phase sandwich ELISA kit from Biodata International. Plasma concentrations of IL-18, sICAM-1 and sE-selectin were also measured using ELISA kits (Bender Medsystems Diagnostics). Plasma nitrotyrosine levels were assessed using a commercially available sandwich ELISA assay kit from Oxis Research. Values for sICAM-1 and
Measurement of plasma 8-isoprostaglandin F<sub>2α</sub> and TBARS (thiobarbituric acid-reactive substances)

To evaluate systemic oxidative stress, plasma levels of 8-isoprostaglandin F<sub>2α</sub> and TBARS were determined. Plasma 8-isoprostaglandin F<sub>2α</sub> was measured by an ELISA using the Biotech 8-isoprostane assay kit (Oxis International) after extraction by passage through a C<sub>18</sub> Sep-Pak cartridge. Plasma 8-isoprostaglandin F<sub>2α</sub> values were expressed as pg/ml. Plasma TBARS were measured colorimetrically based on a method described previously [24]. TBARS values were expressed in nmol/ml MDA (malondialdehyde) equivalents.

Measurement of serum hs-CRP (high-sensitivity CRP)

Serum hs-CRP was measured using the Dade Behring N Highly Sensitive CRP assay (Dade Behring Diagnostics) on the BN ProSpec. This assay utilizes a monoclonal antibody coated to polystyrene particles and fixed-time kinetic nephelometric measurements. The calibration curve is generated from multiple dilutions of a human calibrator traceable to the international reference preparation for proteins in human serum 91/0619 (CRM 470). The BN ProSpec nephelometer makes a 1:20 dilution to measure CRP concentrations between 0.18 and 11.0 mg/l, and a 1:100 dilution above 11.0 mg/l.

Statistical analysis

Results are means ± S.E.M. Comparisons between control and pre-treated groups were assessed using ANOVA with the group as the main effect. To compare the patient group at baseline with post-treatment, a two-way ANOVA with drug and time as main effects was used. Time was used for the repeated measures on each patient. The interaction of drug by time was tested at a 0.05 level and was dropped from the model because no significant differences were detected. To evaluate whether pre-existing effects influence results multivariate analysis and correlations using potential continuous covariates (age, heart rate, systolic blood pressure and diastolic blood pressure) or independent sample Students’ t test for normal covariates (gender, hypertension, diabetes and drug use) were performed. P < 0.05 was considered significant. The SAS system program was used for statistical analysis.

RESULTS

Study population

Thirty-eight patients were randomized. Of these, nine patients did not complete the 30-day follow-up. Five patients died (two patients from terminal CHF, two patients from lethal arrhythmia and one patient from stroke), two patients failed to stabilize on inotrope support for at least 24 h and two patients underwent cardiac transplantation. Thus 29 patients completed the study. The clinical characteristics of the study group are shown in Table 1. The healthy control subjects (n = 15; ten men and five women) were slightly, but not significantly (P = 0.08), younger than the patient population (54.1 ± 3.2 compared with 61.9 ± 2.6 years). Patients had heart failure for 62 ± 88 months (median, 36 months). LVEF (left ventricular ejection fraction) was severely depressed, consistent with advanced disease. Patients had ongoing worsening symptoms for 12 ± 2.3 days prior to admission. The majority of patients studied had CHF caused by ischaemic heart disease. Of the patients studied, 45% were hypertensive or diabetic. Mean plasma creatinine at the time of admission was 149 ± 51 μmol/l (median, 142 μmol/l). The majority of patients (67%) were treated with either an ACE (angiotensin-converting enzyme) inhibitor and/or an ARB (angiotensin receptor blocker). Most of the patients were treated with diuretics and digoxin, and 38% received spironolactone (25–50 mg/day) on a chronic basis. During the trial, the dose of furosemide increased from 106 ± 17.4 at baseline to 146 ± 6.1 mg/day at 30 days (P = 0.015).

Changes in functional class assessed using the NYHA (New York Heart Association) classification before the current episode of decompensation, at the time of admission during the phase of decompensation and at the

<table>
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<th>Table 1 Baseline clinical characteristics of the patient study population</th>
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<td>Aetiology (n)</td>
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| Ischaemic heart disease                      | 21 (73%)
| IDC                                          | 2 (7%)
| Others                                      | 6 (21%)
| Drugs (n)                                    |
| ACEI                                        | 11 (38%)
| ARB                                         | 11 (38%)
| Diuretics                                   | 25 (86%)
| Spironolactone                               | 11 (38%)
| Digoxin                                     | 19 (66%)
| β-Blockers                                  | 7 (24%)
| Hypertension (n)                             | 13 (45%)
| Diabetes (n)                                | 13 (45%)

Data are means ± S.E.M., or numbers. Drugs represent medication used chronically prior to the admission. IDC, idiopathic dilated cardiomyopathy. ACEI, ACE inhibitor.
end of the study are shown in Figure 1. Of the patients studied, 52% exhibited class III symptoms on a chronic basis, and they all presented with advanced NYHA class III–IV at the time of admission. A proportion of patients (n = 12; 42%) presented with heart failure with features of balanced low output and fluid retention, 38% (n = 11) presented with findings consistent with predominantly low cardiac output, and 20% (n = 6) had marked fluid retention. Seventeen patients (59%) had heart failure worsened by no apparent reasons, whereas seven patients (27%) had heart failure caused by either non-compliance to fluid or salt restriction, overexertion, or exposure to warm temperature. The remaining five patients had heart failure symptoms worsened by uncorrected valvular disease (n = 2), β-blockers (n = 2) or following treatment for bronchitis (n = 1). The functional class significantly improved at 30 days, with 97% of patients presenting either class II (34.5%) or class III (62.1%) NYHA symptoms (P < 0.005) following treatment (Figure 1).

Heart rate (82 ± 15 compared with 75 ± 15 beats/min) and systolic blood pressure (66.4 ± 11.7 compared with 60.4 ± 8.5 mmHg) decreased significantly (P < 0.05) at 30 days compared with at admission, consistent with an improvement in clinical status. There were no significant differences in these parameters between dobutamine- and milrinone-treated groups (results not shown).

**Six-min walk test**

Patients exhibited an improvement in the functional status as measured by the 6-min walk test at 30 days compared with at admission (240 ± 94 compared with 317 ± 82 m respectively; P < 0.05). There were no differences between dobutamine- and milrinone-treated groups.

### Plasma cytokines and chemokines

As shown in Table 2, plasma levels of IL-6 and IL-18 were significantly increased (P < 0.001) in patients on admission compared with healthy control subjects. Although IL-6 was increased 2-fold above control values, IL-18 was increased 4–5-fold. At 30 days after treatment, plasma IL-6 and IL-18 decreased significantly (P < 0.05). There were no differences in responses between milrinone- and dobutamine-treated groups (results not shown). MCP-1 was not increased in patients with CHF and treatment had no effect on plasma MCP-1 levels.

### Plasma levels of soluble adhesion molecules

Plasma levels of sICAM-1 and sE-selectin were markedly elevated in patients with decompensated CHF compared with healthy controls (Table 2). Inotropic therapy significantly decreased sICAM levels compared with levels on admission. Plasma concentrations of sE-selectin were not influenced by therapy.

### Circulating markers of oxidative stress

Plasma concentrations of MDA (TBARS), 8-isoprostaglandin F2α (8-isoprostane) and nitrotyrosine were significantly increased in patients on admission compared with controls (Table 2). Following treatment, levels of markers of oxidation were significantly reduced, but were not completely normalized.
Plasma hs-CRP

Plasma levels of hs-CRP were significantly increased \((P < 0.01)\) in patients with CHF compared with controls. CRP decreased significantly \((P < 0.05)\) at 30 days. There were no differences between milrinone- and dobutamine-treated groups (results not shown).

Correlation studies

To evaluate whether pre-existing conditions contribute to changes associated with CHF (baseline and treated), we performed multivariate analysis and correlation studies with potential continuous covariates (age, heart rate and blood pressure) or independent sample Student \(t\) tests for nominal covariates (gender, hypertension, diabetes and drug use). The only significant correlation between inflammatory markers and continuous covariates was for 8-isoprostaglandin \(F_2\alpha\) and heart rate \((r = 0.46, P = 0.02)\). Neither gender, hypertension nor diabetes influenced the results.

DISCUSSION

Major findings from the present study demonstrate that (i) irrespective of the aetiology, CHF is associated with an inflammatory process and enhanced oxidative stress, (ii) short-term inotropic therapy improves clinical status as assessed by the NYHA classification and the 6-min walk test, predictive of mortality and morbidity in CHF [23], and (iii) recompensation is associated with reduced plasma levels of inflammatory mediators and oxidative markers. From the present study we cannot confirm whether the beneficial anti-inflammatory/antioxidant effects of treatment are due to a direct action of inotropic agents or a consequence of improved cardiac function secondary to treatment. Nevertheless, what is important is that recompensation mediated by short-term inotropic support was associated with reduced inflammation/oxidative stress. These findings are clinically relevant because elevated levels of biomarkers of inflammation and oxidation are related to adverse effects on myocardial structure and function and poor prognosis in patients with CHF [7,21,25–27]. Processes influencing inflammation/oxidation in patients with CHF may relate to associated risk factors [26,28]. Many of the patients studied had a history of ischaemic heart disease and 45% had diabetes or hypertension, which are themselves pro-inflammatory states [16,29–32]. To evaluate whether baseline changes in inflammatory/oxidation markers may be due to pre-existing factors and to assess whether treatment effects on inflammation are indeed related to inotrope intervention, we performed a combination of statistical tests, including multivariate analysis, correlation studies and independent Student \(t\) testing. Using this approach, except for a positive correlation between heart rate and plasma 8-isoprostaglandin \(F_2\alpha\) levels, the clinical relevance of which remains unclear, we did not find any meaningful significant associations. Hence our findings suggest that CHF is most probably responsible for increased baseline inflammatory/oxidative factors and that treatment effects are most probably related to inotrope intervention.

Our results of elevated concentrations of IL-6 and hs-CRP in patients with CHF confirm those of others [29]. Unlike previous investigations [31], we did not find MCP-1 to be elevated. Reasons for this are unclear; however, patients in the present study exhibited a rapid deterioration as opposed to a more chronic and stable state in previous reports, making direct comparisons of our observations with those of others difficult.

IL-18, a member of the IL-1 family, is a pro-inflammatory cytokine with multiple biological functions [33]. IL-18, originally named as IGIF (interferon-\(γ\)-inducing factor), induces TNF\(α\) and IL-6 and is expressed in vascular endothelial cells and macrophages in human heart. IL-18 has been demonstrated to be one of the strongest predictors of cardiovascular death in stable and unstable angina [32]. However, little is known about IL-18 status in patients with CHF. In our present study we demonstrate for the first time that plasma IL-18 is markedly elevated. Importantly, all of the biomarkers studied in the present paper, the magnitude of increase above control levels was highest for IL-18. This may be explained, in part, by the fact that most patients had ischaemic heart disease and, consequently, ongoing silent ischaemia, known to be associated with increased IL-18 [33,34].

Adhesion molecules, which regulate leucocyte migration into the vascular wall, have been implicated in atherosclerosis, thrombosis, allograft rejection post-transplantation and restenosis following coronary angioplasty [16,20,35,36]. However, their significance in CHF is less well defined. Increased expression of sICAM-1 and integrin CD11a/CD18 (lymphocyte function-associated antigen-1) as well as macrophages expressing TNF\(α\) were demonstrated in myocardium of patients with CHF [5,21]. Since TNF\(α\) up-regulates expression of adhesion molecules, this may contribute to macrophage infiltration and inflammation in the myocardium. Another mechanism for adhesion molecule up-regulation could be through ROS, which are known to stimulate adhesion molecule production, and which are increased in failing myocardium [15,33,36]. Plasma sICAM and sE-selectin were significantly elevated in patients with CHF in our present study. Since soluble forms of adhesion molecules originate from proteolytic cleavage of cell-membrane-bound molecules, they serve as important markers of cellular activation and inflammation [37,38]. We cannot differentiate the origin of increased circulating adhesion molecules in our patients, but both cardiac and vascular sources may be important. This is particularly relevant in our patient cohort, because many
had ischaemic heart disease and hypertension, which are associated with increased cardiac and vascular inflammation. Our findings of increased adhesion molecules in CHF support other studies, which suggested that ICAM-1 may be an important prognostic indicator in CHF, since levels of ICAM-1 increase with increasing severity of failure [21,25]. Increased VCAM-1 (vascular cell-adhesion molecule-1), E-selectin and P-selectin have also been demonstrated, which could be a manifestation of systemic inflammation in CHF [21,25].

Increased generation of ROS has been reported in experimental and human CHF and has been associated with activation of inflammatory transcription factors, up-regulation of adhesion molecules, cardiac apoptosis and fibroblast proliferation, important processes involved in cardiac remodelling in CHF [38]. In addition, oxidative stress has detrimental effects on cardiac function since oxygen free radicals depress excitation–contraction coupling [39]. We measured three different markers of oxidative excess: TBARS and 8-isoprostaglandin F(_2_α), indices of lipid peroxidation, and serum nitrotyrosine, an indicator of tyrosine nitrosylation of proteins, a redox-sensitive process. All three markers were significantly elevated in patients with CHF, confirming that oxidative stress is increased systemically in CHF. Interestingly, the magnitude of increase above control values (1.5–2-fold) was similar for the three parameters, indicating consistency of different oxidative processes in CHF.

An important novel finding was the significant decrease in plasma levels of inflammatory/oxidative markers following short-term inotropic treatment. The 30-day time point was selected to allow sufficient time for clinical stabilization and restoration of cardiopulmonary homoeostasis, as reported previously [40]. Similar time points were selected in clinical trials such as EMOTE (EnoxiMone in intravenous inOTropE) investigating the effect of enoximone on the success of weaning chronic heart failure patients with advanced heart failure [40]. At 30-days post-therapy, all patients exhibited marked improvement in cardiac function, as evidenced by the improvement from NYHA class IV pre-admission to NYHA class II–III post-treatment and by the 6-min walk test. This was associated with reduced plasma levels of IL-6, IL-18, TBARS, 8-isoprostaglandin F(_2_α), sICAM and nitrotyrosine. Milrinone and dobutamine had similar effects and accordingly data in the treated groups were pooled.

In the present study, we cannot differentiate whether favourable effects of inotropes are direct drug-related actions [41,42] or secondary phenomena to improved cardiopulmonary and peripheral function. However, what is significant is that the inflammatory/oxidative response was reduced, and a decrease in some of these markers has been associated with improved prognosis and outcomes in CHF [43–45]. Amelioration of inflammatory processes and oxidative stress following short-term inotropic treatment may contribute to improved cardiac function in patients with CHF. Further in-depth investigations on isolated failing myocardium from patients will be necessary to determine the exact role of inotropes, immunomodulation, inflammatory activation and oxidative stress in the pathophysiological processes associated with CHF. Moreover, larger long-term follow-up studies will be necessary to evaluate whether changes in serum markers of inflammation and oxidation associated with or without inotropic therapy do in fact reflect outcomes.

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