Adjunctive therapy with low-molecular-weight heparin in patients with chronic heart failure secondary to dilated cardiomyopathy: One-year follow-up results of the randomized trial

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Background  Defective endothelial function has been shown in dilated cardiomyopathy. Therefore, improvement in endothelial function after low-molecular-weight heparin (LMWH) therapy may be clinically beneficial. Consequently, the effect of adjunct enoxaparin, a LMWH, on standard treatment of dilated cardiomyopathy was investigated.

Methods  This was a randomized, standard treatment–controlled, 2-center pilot trial of 102 patients (52 receiving adjunctive therapy with enoxaparin at a dosage of 1.5 mg/kg daily for 3 months and 50 receiving standard therapy with angiotensin-converting enzyme inhibitors, β-blockers, and diuretics alone) with stable chronic heart failure secondary to dilated cardiomyopathy (New York Heart Association [NYHA] class II and III; left ventricular [LV] ejection fraction, ≤40%). All patients underwent coronary angiography and endomyocardial biopsy and were clinically stable for at least 6 months before enrollment. The combined primary end point included mortality, urgent heart transplantation, and readmission to hospital due to heart failure progression. The secondary end point was to determine the severity of heart failure (serum level of N-terminal brain natriuretic peptide), cardiac function (LV ejection fraction by radionuclide ventriculography), LV diameters by echocardiography, exercise capacity (changes in NYHA class, changes in peak oxygen consumption), and changes in quality of life (Minnesota Living with Heart Failure questionnaire). The clinical outcome was assessed after 6 and 12 months of therapy.

Results  Baseline characteristics were comparable in both groups. Five patients dropped out during 12 months of the study. Twelve patients achieved primary end point (8 in the control group and 4 in the LMWH group). The free survival rate was 94% for the LMWH group and 90% for the controls (not statistically significant). After the 12-month period, in the LMWH group, N-terminal brain natriuretic peptide level and LV diameters decreased significantly (P < .001 and P = .006, respectively), whereas LV systolic function increased (P < .001). Changes in exercise capacity and subjective improvement did not differentiate the groups (nonsignificant). Adverse reactions to the enoxaparin therapy were minor and transient.

Conclusions  In patients with chronic heart failure due to dilated cardiomyopathy, adjunct long-term enoxaparin therapy may offer additional clinical benefit without deleterious effects on major cardiac events. (Am Heart J 2006;152:713.e1–713.e7.)

Systolic heart failure may be viewed as a progressive disorder initiated by different events and sustained by multifaceted pathophysiologic mechanisms. Regardless of the nature of the initiating events and optimized therapy used, the disease progresses. The altered state of hemostasis and abnormalities of the endothelial function had been previously postulated among mechanisms involved in perpetuating myocardial failure.1,2 In addition, the poor systolic function may also cause blood stasis within a dilated left ventricle, perpetuating the flow disturbances as well.3,4 Thus, it may be hypothesized that the improvement in microvascular flow due to antithrombotic and anti-inflammatory...
properties of heparin may reduce microvascular ischemia and improve the clinical status of patients with heart failure. 5,6

The purpose of this study was to assess the safety, tolerability, and clinical efficacy of a long-term treatment with therapeutic doses of enoxaparin in patients with stable chronic heart failure secondary to dilated cardiomyopathy, who were enrolled in the prospective, randomized study.

Methods

Patient population

From January 2003 to April 2004, 102 of 241 consecutive patients screened with unexplained chronic heart failure due to dilated cardiomyopathy (New York Heart Association [NYHA] class II and III, left ventricular [LV] ejection fraction ≤40%) were entered into the trial. The patients were randomized in a 1:1 ratio of enoxaparin to conventional therapy alone by an external investigator who was blinded to the drug. However, the study was open-labeled. For at least 3 months before enrollment, all patients had to remain clinically unchanged; patients were on the same drug regimen that included angiotensin-converting enzyme (ACE) inhibitor (captopril, 75-150 mg/d) or angiotensin II receptor blockers (2.5% of patients), β-blockers (metoprolol CR, 50-100 mg/d, or carvedilol, 50-75 mg/d), and diuretics (spironolactone, 50 mg/d, and furosemide, 40 mg/d). Therapy with digitalis (71% of patients), antiarrhythmic drugs (amiodarone, 200-400 mg/d; 8% of patients), and antiplatelet drugs was allowed but was not required. In addition, during the study only minor changes in medications or dosages were permitted.

Exclusion criteria included blood coagulation disorders, internal bleeding, a history of heparin-associated thrombocytopenia, platelet count <100 × 10⁹/L, improvement in clinical status on conventional therapy in the outpatient period preceding hospitalization, any changes narrowing epicardial coronary arteries in coronary angiography, type 1 diabetes mellitus, previously documented history of hypertension, valvular heart disease (except the relative mitral regurgitation), and endocrine diseases. Patients with significant renal and liver diseases and drug or alcohol abuse problems and those who had undergone steroid or heparin therapy within 3 months before screening were also excluded from the study. The study was approved by the local review board, and all study patients gave written informed consent.

Study design

This was a 3-month, randomized, standard treatment-controlled, and 2-center pilot trial with 6- and 12-month follow-ups to confirm the hypothesis that long-term therapy with a therapeutic dose of enoxaparin would modify the clinical outcome of patients with chronic heart failure due to dilated cardiomyopathy. The follow-up ended in May 2005. Patients received either weight-adjusted enoxaparin (1.5 mg/kg) subcutaneously at 12-hour intervals within 14 days following by its daily administration up to 3 months or the conventional therapy alone. They were followed up over a period of 6 and 12 months.

Study procedures

Clinical background information was obtained, and physical examination, resting electrocardiogram, 24-hour Holter monitoring, blood chemistry data, and 2-dimensional and M-mode echocardiography were performed in all patients at baseline and follow-ups. Echocardiography was performed with a 2.5-MHZ phased-array probe (GE Vingmed Ultrasound, System V, Milwaukee, WI) by 2 investigators blinded to the clinical status of the patients. All echocardiographic images were acquired in the standard views as recommended by the American Society of Echocardiography Committee. Special attention was paid to the following parameters: LV contractility (regional and global), LV diastolic volume, LV diastolic and systolic diameters, and mitral regurgitation severity. The interobserver variability of the above-mentioned echocardiographic parameters was <3.1%. LV ejection fraction was assessed by radionuclide ventriculography with technetium Tc 99m. The NYHA classification ranking was used to classify functional capacity. The subjects completed a Minnesota Living with Heart Failure Quality of Life (MQL) questionnaire (scale, 0-105) to verify their subjective improvement. Maximal treadmill test (Marquette Electronics Inc, Milwaukee, WI) was done, with metabolic measurement as peak oxygen consumption obtained by breath-by-breath analysis of expired gas (Ve max, SensorMedics Corp, Yorba Linda, CA) was determined to assess severity of heart failure. Indexed V O₂ consumption (%) was calculated as peak oxygen consumption divided by maximal predicted oxygen consumption. Before enrollment, all patients underwent coronary angiography and right ventricular endomyocardial biopsy.

Laboratory assays

Complete laboratory analyses were performed at baseline and at 6- and 12-month follow-ups. Collected blood samples were immediately chilled to 4°C, centrifuged, and analyzed immediately or stored at −80°C when required. N-terminal brain natriuretic peptide (NT-proBNP) level was measured by using a commercially available kit from Roche Diagnostics (Mannheim, Germany) on an Elecsys 2010 analyzer with analytical sensitivity of <5 pg/mL (upper limits of normal of 100 pg/mL in men and 150 pg/mL in women as proposed by the manufacturer). Plasma concentrations of von Willebrand factor (reference range, 50%-160%) and D-dimer (normal concentration, <0.5 µg/mL) were measured by using an STA Compact analyzer (STA Lyse t vWF, Diagnostica Stago/Roche, Asnieres, France) with a coefficient of variation of ≤5% and ≤10%, respectively. The thrombin-antithrombin III complexes were detected in plasma by Enzygnost TAT ELISA (Dade Behring, Marburg, Germany) (reference range, 1.0-4.1 µg/L; coefficient of variation ≤6%).

Endomyocardial biopsy, histology, and immunohistology

A minimum of 6 specimens (4 for histology, 2 for immunohistology) was obtained from the right ventricular septum with a biopette (Cordis Corp, Miami, FL). The histologic examination was performed by 2 investigators independently and was assessed according to the Dallas criteria (interobserver variability, 2.6%). For immunohistochemistry, frozen sections were incubated with murine monoclonal
Table I. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 50)</th>
<th>LMWH group (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>37 (±12)</td>
<td>39 (±11)</td>
</tr>
<tr>
<td>Sex, n, male/female</td>
<td>44/6</td>
<td>43/9</td>
</tr>
<tr>
<td>Type II diabetes mellitus, n</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>ICD therapy, n</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Duration of heart failure symptoms (y)</td>
<td>2.1 (±1.0)</td>
<td>1.9 (±1.2)</td>
</tr>
<tr>
<td>NYHA class, n, II/III</td>
<td>46/4</td>
<td>44/8</td>
</tr>
<tr>
<td>Conduction disorders, n</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left bundle branch block</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>86 (±21)</td>
<td>84 (±14)</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>29.8 (±6.8)</td>
<td>27.8 (±7.2)</td>
</tr>
<tr>
<td>LV diastolic dimension (mm)</td>
<td>67.6 (±6.2)</td>
<td>68.5 (±8.7)</td>
</tr>
<tr>
<td>LV systolic dimension (mm)</td>
<td>51.6 (±7.9)</td>
<td>55.1 (±11.1)</td>
</tr>
<tr>
<td>LV diastolic volume (mL)</td>
<td>204.4 (±55.9)</td>
<td>216.1 (±89.8)</td>
</tr>
<tr>
<td>Mitral regurgitation grade</td>
<td>1.3 (±0.7)</td>
<td>1.3 (±0.9)</td>
</tr>
<tr>
<td>Peak V̇O₂ (mL·kg⁻¹·min⁻¹)</td>
<td>16.2 (±4.1)</td>
<td>15.7 (±5.2)</td>
</tr>
<tr>
<td>MOL score</td>
<td>38 (±21)</td>
<td>39 (±19)</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL), median</td>
<td>960.3</td>
<td>1125</td>
</tr>
<tr>
<td>von Willebrand factor, median</td>
<td>(294.5-2835.5)</td>
<td>(394.9-3750.5)</td>
</tr>
<tr>
<td>D-dimer, median</td>
<td>0.22 (0.22-0.24)</td>
<td>0.23 (0.22-0.54)</td>
</tr>
<tr>
<td>Thrombin-antithrombin III complex, median</td>
<td>3.61 (1.70-3.92)</td>
<td>3.85 (3.07-4.87)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.97 ± 0.23</td>
<td>0.91 ± 0.23</td>
</tr>
<tr>
<td>Dallas classification (active/ borderline/no myocarditis), n</td>
<td>0/2/48</td>
<td>0/1/51</td>
</tr>
</tbody>
</table>

Values are expressed as mean (±SD) or as indicated.

antihuman antibodies: anti-HLA class II (DR antigens), α chain (clone TAL1B5), anti-HLA class I (ABC antigens, clone W6/32), anti-CD5 for T lymphocytes (clone T3-4B5), antiamphocytes (clone EBM11), anti-von Willebrand factor (clone F8/86), and anti–membrane-bound terminal complement complex (C5b-9, clone aE11), all from DAKO A/S (Glostrup, Denmark). The bound primary antibody was detected by using the EnVision method (DAKO EnVision Kit). The 2 blinded investigators evaluated each specimen evaluated qualitatively, semiquantitatively (expression index based on semiquantitative scale from 0 to +), and quantitatively (counts from at least 10 high-power fields under 400× magnification) with interobserver variability <1.9%.

Outcome measures and definition of improvement

The primary objective of this study was to determine the composite end point of death, urgent cardiac transplantation, and readmission to hospital due to heart failure progression at 12 months of follow-up. The secondary objective was to determine severity of the disease (serum level of NT-proBNP, LV ejection fraction by radionuclide ventriculography), LV diameters and LV diastolic volume by echocardiography, exercise capacity (changes in NYHA class, changes in peak oxygen consumption), and changes in MQL score. In addition, selected laboratory analyses were compared at baseline and after 6 and 12 months of therapy. In addition to these outcome measures, the patients were classified as improved if they had a decrease in NT-proBNP levels of at least 50% as compared with baseline measures, together with an increase of >5 percentage points in the absolute ejection fraction.

Statistical analysis

Using a 2-sided test (α = 0.05, β = 0.10), 50 patients were considered to be necessary to detect significant differences between the groups in the left ventricular ejection fraction and NT-proBNP level. The primary analysis was performed for a 12 month end point according to the intention-to-treat principle including all randomized patients (except patients who were withdrawn) by log-rank test for event-free survival analysis. A prespecified secondary analysis was designed to assess the true effect of the strategy, tested by including only patients who achieved 6- and 12-month follow-ups. Categorical variables were analyzed by χ² statistics, but continuous variables were analyzed by Student t test for between-group comparisons and 2-way (group effect and time effect) repeated-measures analysis of variance. Normally distributed data are presented as mean ± SD, but nonnormally distributed data are presented as median with an interquartile range (IQR; 25th and 75th percentiles). Differences were considered statistically significant when P < .05. The statistical analysis was performed with the SPSS version 13.0 software package (SPSS, Inc, Chicago, IL).

Results

Patient population, safety, and tolerability

A total of 102 patients underwent randomization. As shown in Table I, there was no significant difference in baseline demographic or clinical characteristics between the groups. In addition, there was no statistically significant difference at baseline between both groups with respect to histologic and immunohistologic panel studied. For the carry-forward analysis, 5 patients (2 in the control and 3 in the LMWH group) did not finish the trial for the following reasons: incorrect study drug administration (1 in the control and 2 in the LMWH group) and poor compliance (1 in the control and 1 in the LMWH group). All these patients were still alive at the end of the study (data collected by telephone). The final clinical assessment based on carry-forward principle was performed on 85 subjects (83.3%), excluding patients who achieved primary efficacy end point results (n = 12), as reported below. Adverse reactions to the enoxaparin therapy such as injection hematomas were minor and transient. No other side effect was found in the enoxaparin-treated patients.
Primary efficacy end point results

As shown in Table II, the composite end point of death, urgent heart transplantation, and readmission to hospital was less common in patients treated with enoxaparin as compared with the control; however the difference was not statistically significant (NS). The free survival rate after the 12 months of follow-up was 90% for the control subjects and 94% for the enoxaparin-treated patients (4 deaths in the control and 1 death in the LMWH group, all sudden cardiac deaths in origin; log-rank test, NS). One patient from the control and 2 patients from the LMWH group underwent urgent heart transplantation. Readmission to hospital due to heart failure collapse was found in 4 cases (3 from the control and 1 from the LMWH group). In summary, the composite primary end point was achieved by 8 conventionally treated patients and 4 enoxaparin-treated subjects (NS).

Secondary efficacy end point results

The results are presented in Table II. For the carry-forward analysis, 85 patients (83.3%) finished the 12-month period under the study medication. In these patients, long-term clinical efficacy of therapy was determined. Accordingly, only a few outcomes showed a benefit of enoxaparin therapy. There was no significant difference between the groups in the plasma NT-proBNP levels. However, the adjunctive enoxaparin therapy was associated with a significant decrease in NT-proBNP levels after 6 and 12 months of therapy as compared with the baseline data (Figure 1). LV ejection fraction increased significantly in the enoxaparin-treated patients after 6 months of follow-up and it was maintained up to the 12th month of follow-up with significant differences between groups after the end of the study (95%...
CI, 1.01-8.17; \( P = .023 \); Figure 2). The remaining secondary end points (LV systolic and diastolic diameters, diastolic volume, and NYHA functional class) did not differ between the groups. With regard to quality of life, MQL score declined at the study end point by an average of 3 points (from 39 \( \pm \) 19 to 36 \( \pm \) 22), indicating only a trend to subjective improvement in the enoxaparin-treated subjects as compared with the baseline data (from 38 \( \pm \) 21 to 37 \( \pm \) 23) (\( P = .055 \)).

Over the 12 months of the study, there was no significant change either within or between the treatment groups in the maximal exercise capacity as measured by \( V' \) \( O_2 \). At baseline, the median plasma level of thrombin-antithrombin III complex, being a prothrombotic state marker, exceeded those found in healthy subjects. At 12 months, however, the thrombin-antithrombin III complex levels decreased significantly in the enoxaparin-treated patients as compared with baseline data (\( P = .001 \)), with a statistically significant difference between-group comparison (\( P = .003 \)). The plasma levels of D-dimer, which reflect fibrinolytic activity, did not exceed upper levels found in clinically healthy subjects at 12 months, however, the thrombin-antithrombin III complex levels decreased significantly in the enoxaparin-treated patients as compared with baseline data (\( P = .001 \)), with a statistically significant difference between-group comparison (\( P = .003 \)).

Protocol-specified definition of improvement

Based on the protocol-specified definition, 25 (50%) of 50 patients in the LMWH group and 7 (14.9%) of 47 patients in the control group met the criteria of improvement (\( P < .001 \)) at 6 months. After 12 months, such improvement was reported in 25 (50%) of 50 versus 12 (25.5%) of 47 patients (\( P = .013 \)), respectively.

Discussion

To the best of our knowledge, this is the first study to demonstrate that a long-term treatment with therapeutic doses of enoxaparin was safe and well tolerated in patients with chronic heart failure secondary to dilated cardiomyopathy. As far as the clinical improvement is concerned, no significant differences in the majority of clinical outcomes were present between the enoxaparin- and standard-treatment groups. However, the improvement in clinical status in the LMWH-treated group was achieved at the time of the follow-ups as compared with the baseline data. It was corroborated by the improvement in endogenous cardiac hormonal system imbalance as reflected by the decrease in NT-proBNP levels in the enoxaparin-treated patients. Accordingly, it may be suggested that standard heart failure therapy with adjunction of LMWH may additionally offer clinical benefit for patients with chronic heart failure, although significant decrease in major cardiac events in the enoxaparin-treated patients has not been achieved in this trial.

It is clear that LMWH possesses many biologic activities besides retarding blood coagulation including anti-inflammatory effects on complement activation and neutrophil-platelet interactions.\(^7\) In addition, LMWH affects the immune response\(^8\),\(^9\) and alters the architecture of the extracellular matrix by influencing biosynthesis of collagen and proteoglycans.\(^10\) Moreover, as indicated by the studies of Frizelle et al, heparin treatment can reduce myocardial inflammation and decrease collagen deposition in an animal model of myocarditis.\(^11\) Taking into account that a low grade of inflammation was postulated in patients with heart failure,\(^12\),\(^13\) the anti-inflammatory activities of LMWH might influence clinical outcomes in these patients.

Considering the pleiotropic effects of LMWH in the context of the current study, special attention should be paid to the ability of enoxaparin to enhance tissue factor pathway inhibitor release and subsequent nitric oxide production.\(^5\) It is postulated that microvascular dysfunction plays a significant role in heart failure development and its progression due to the impaired endothelial function.\(^2\),\(^14\),\(^15\) In addition, during hypoxia (ischemia) the microvascular endothelium partially loses its natural anticoagulant properties especially in a subendocardial layer.\(^16\),\(^17\) Thus, the improvement in the endothelium-dependent vasodilatation due to heparin therapy would decrease the microvascular ischemia and may have clinical benefits in the enoxaparin-treated patients. In summary, the pleiotropic effects of LMWH may work in concert with its anticoagulant activity to provide the clinical benefit that was observed after
enoxaparin administration to those patients who had percutaneous coronary intervention in the Thrombolysis In Myocardial Infarction and Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-Wave Coronary Events trials. 18

In accordance with previous reports, 19,20 we observed a clinically detectable tendency to hypercoagulability in the randomized patients, although only a few of them presented thrombin-antithrombin levels exceeding the upper normal limit level found in healthy subjects. However, we did not observe a significant increase in plasma D-dimer or von Willebrand factor levels as was previously reported in patients with heart failure. 19,21

The discrepancy may be because most our patients had been in a stable clinical condition for a long time. Previous investigations studied patients with recent (acute) onset of heart failure or did not specify the duration of heart failure symptoms or included patients with symptomatic congestive heart failure. 19 Moreover, therapy with ACE inhibitors may influence the plasma activity of serum markers of coagulation system as previously reported by Gibbs et al. 22 Such improvement in a hypercoagulable state after a short-term therapy with LMWH in patients with heart failure was also reported. 23

Study limitations

There are a number of study limitations. The major limitation of this study was that this trial was not blinded or placebo controlled. However, thus far there have been no data concerning tolerability or safety of long-term therapeutic doses of LMWH. Therefore, taking ethical aspects into account, we decided to do an open-label study. On the other hand, all assessments were completed by investigators entirely blinded to the patients’ treatment assignments. It must be emphasized that the randomized patients are not representative of all subjects with heart failure because of the exclusion of the patients with ischemic etiology of heart failure and the relatively young population studied. In addition, most of the studied patients presented with low postheparin bleeding risk. The calculation of the power study was based on secondary efficacy end points instead of primary efficacy end point results. Bearing in mind our previous pilot studies and patients’ stable condition before enrollment, we would find it difficult to await a high incidence of major cardiac events within the 12-month therapy in such cohort of patients. However, the lack of differences between the groups in primary efficacy end point results should be taken with caution. With the current results into consideration, a large multicenter and randomized placebo-controlled trial is warranted. Additional studies are also warranted to assess the effect of LMWH therapy in patients with more advanced heart failure and patients with ischemic heart failure.

Conclusions

These studies provide the evidence for the safety and well tolerability of long-term efficacy of adjunctive therapy with LMWH to patients with heart failure secondary to dilated cardiomyopathy. Thus, such a therapy may be effectively administered for a long time in an ambulatory self-application and controlled manner. However, complications and the inconvenience of a daily subcutaneous administration of this drug should be taken into account. Relatively small clinical benefit observed in LMWH therapy as compared with conventional therapy alone needs further investigation in a large cohort of patients. However, it seems that despite a lack of spectacular benefits of adjunctive therapy with LMWH in stable heart failure, such a therapeutic strategy may provide a new insight into therapeutic approaches for salvaging the myocardium.

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References