Angiogenic Effects of Low Molecular Weight Heparin in Patients With Stable Coronary Artery Disease: A Pilot Study

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**Objectives.** The study was designed to assess the feasibility of conducting a trial to investigate whether exercise and low molecular weight heparin therapy with dalteparin sodium (Fragmin) would improve collateral function to the ischemic myocardium in patients with coronary artery disease.

**Background.** The severity of myocardial ischemia in patients with coronary artery disease is at least partly dependent on the status of the collateral circulation. Therefore, improvement in collateral function would potentially provide a unique way of alleviating myocardial ischemia. Because the combination of ischemia and heparin has previously been demonstrated to enhance collateral growth, we studied the anti-ischemic effects of combined treatment with dalteparin sodium and exercise-induced ischemia in patients with coronary artery disease.

**Methods.** Twenty-three patients with stable coronary artery disease were randomized to receive either subcutaneous dalteparin sodium or placebo for a 4-week period. Patients received either placebo or 10,000 IU of dalteparin sodium by subcutaneous injection once daily for weeks 1 and 2 and 5,000 IU daily for weeks 3 and 4. During the 1st 2 weeks, patients were exercised to ischemia three times a day. At baseline and 4 weeks after treatment, treadmill exercise testing, exercise radionuclide ventriculography, and 48-h ambulatory ST segment monitoring were performed.

Myocardial ischemia is often inadequately relieved by conventional therapy in patients with coronary artery disease. Because the magnitude of myocardial ischemia is related to oxygen demand, the severity of the coronary stenoses and status of the collateral circulation subserving the ischemic myocardium, it is likely that enhancement of collateral function not only would potentially provide a unique way of relieving myocardial ischemia and ameliorating symptoms, but also would reduce the size of the myocardial infarct zone if infarction occurred as a result of sudden occlusion of the feeder artery.

Sustained ischemia stimulates collateral growth (1-8), which is at least partly due to local release of growth factors (5). Heparin potentiates the activity of growth factors (9-11), and the combination of heparin and ischemia has been demonstrated in canine models and humans to enhance collateral function and growth (12-14).

Low molecular weight, nonanticoagulant fragments of heparin also possess angiogenic properties. Dalteparin sodium (Fragmin) (Kabi Pharmacia AB, Stockholm, Sweden), a low molecular weight heparin fragment, has higher bioavailability, a longer half-life and less risk of bleeding than standard heparin (15-22). We undertook the present pilot investigation to determine the feasibility and potential efficacy of a trial of a combination of subcutaneous dalteparin sodium and exercise-induced ischemia in producing a clinically important improvement in collateral function in patients with stable coronary artery disease. An increase in the rate-pressure product and the duration of exercise at the
onset of myocardial ischemia, a reduction in the number and duration of episodes of myocardial ischemia during daily activities and an improvement in left ventricular function with exercise were prospectively used as indirect indexes of improved collateral function to the ischemic myocardium.

Methods

Patients. Twenty-three patients with stable coronary artery disease, defined as 70% stenosis in one or more major epicardial coronary arteries and evidence of inducible ischemia on exercise testing, were recruited for this study (Table 1). Patients were randomized in a double-blind manner into two groups that received either dalteparin sodium or placebo. Because of the possible variation in the potential for collateral development in different subsets, we stratified patients before randomization into three groups: 1) patients with three-vessel disease, 2) patients who had had coronary artery bypass graft surgery, and 3) the remaining patients without previous bypass surgery and at least one major unobstructed coronary artery. The study was continued until at least 10 patients had been randomized into the two treatment groups. At the end of the investigation, 10 patients were randomized to receive dalteparin sodium and 13 received placebo.

After written informed consent was obtained, all antianginal medications were discontinued for 72 hours before the study period. One patient had a long-standing history of hypertension and was maintained onenalapril therapy throughout the study period. The study was approved by the review body of the National Heart, Lung, and Blood Institute.

Treadmill exercise and drug treatment. At the initial evaluation, patients underwent two maximal treadmill exercise tests between 8 AM and 2 PM. The protocol was selected so that exercise duration before onset of significant (1 mm) ST segment depression was between 4 and 10 min. Nineteen patients exercised using the Bruce protocol and four patients using the combined National Institutes of Health protocol (23). An intra-arterial indwelling catheter was placed in the brachial artery to allow continuous blood pressure measurement and an electrocardiogram was recorded every 30 s during the treadmill test so that an accurate rate-pressure product could be obtained at specified end points.

Patients were randomized to receive either placebo or dalteparin sodium therapy for a 4-week period. They were hospitalized during weeks 1 and 2 and received either placebo or 10,000 IU of dalteparin sodium at 7 AM each morning by subcutaneous injection. After 30 min, all patients underwent three treadmill exercise training periods that were completed within 8 h of the injection. The protocol was individualized so that once 1 mm of ST segment depression developed, the speed and angle of the treadmill were kept constant and patients continued to exercise at the same work load for an additional 5 min. Exercise was terminated at the development of moderately severe chest pain, fatigue, or 10 min after development of 1-mm ST segment depression. This protocol provided three periods/day of sustained myocardial ischemia.

Patients were discharged during weeks 3 and 4, when they self-administered dalteparin sodium or placebo (5,000 IU subcutaneously/day). All patients were sent home without antianginal medications; nitroglycerin was available for pain but not for prophylactic use. After the 2-week outpatient period, patients were readmitted and underwent repeat exercise testing.

The following measurements were made during treadmill exercise testing before and after the 4-week treatment period: the rate-pressure product and duration of exercise at 1) the onset of 1 mm of ST segment depression, 2) the onset of chest pain, and 3) the end of exercise. Mean values from the two testing periods are reported.

Radionuclide ventriculography. Gated equilibrium radionuclide cineangiography was performed at rest and during maximal symptom-limited bicycle exercise at baseline and repeated after 4 weeks at the same peak exercise work load. The left ventricular ejection fraction was determined by computer analysis of the scintigraphic data and regional left ventricular function was assessed subjectively as previously described (23,24). The lower limit for normal rest ejection fraction in our laboratory is 45%.

Ambulatory ST segment monitoring. Before and at the end of the 4-week treatment period, patients underwent ambulatory ST segment monitoring for 48 h during unrestricted normal daily activities out of the hospital and were asked to keep a detailed diary of their symptoms. Bipolar
CMRC leads and modified lead II were monitored and the tapes were analyzed visually at 120 times normal speed, employing the Delmar Avionics model 750A system as previously described (23, 25). An ischemic episode was defined as ≥ 1 mm of ST segment depression that occurred 0.08 s after the J point and was either planar or downsloping and lasted ≥ 1 min. Return of the ST segment to baseline for ≥ 1 min was required between two episodes.

Coagulation measurements. There were no bleeding complications. Partial thromboplastin time increased from 29.2 ± 1.7 s at baseline to 38.2 ± 4.2 s after 4 h and was 29.8 ± 2.2 s at 24 h. Antifactor Xa activity was 0.65 ± 0.1 IU/ml 4 h after and 0.26 ± 0.05 IU/ml 24 h after subcutaneous dalteparin sodium injection.

Statistical analysis. The treatment versus control comparisons for change in continuous variables were assessed parametrically using the Student t test and nonparametrically using a comparison of the proportions improving during the course of the trial (follow-up minus baseline values). The comparison of proportions was made using a chi-square statistic. Among the comparisons, the rate-pressure product at 1-mm ST segment depression during treadmill exercise was deemed before testing to be the single most important determinant of improvement in collateral function. Because the treatment was expected to improve several variables, we performed a multivariate nonparametric analysis, assessing the combined change of a cluster of five variables selected before the study because of their importance as determinants of a change in collateral function. These included: 1) the rate-pressure product at 1-mm ST segment depression during treadmill exercise, 2) the duration of exercise to 1 mm of ST segment depression, 3) the number of episodes of ST segment depression during ambulatory monitoring, 4) the duration of episodes during monitoring, and 5) the change in left ventricular ejection fraction with exercise. The multivariate nonparametric analysis was based on the improvements in each of the five selected variables taken together. These five binomially distributed variables were analyzed using a multivariate analysis of variance. Because the binomial distribution is symmetric and approximated by a normal distribution, a standard statistical multivariate analysis of variance package (SAS Statistical computer package 6.0, SAS Institute) was used. A p value ≤ 0.05 was considered significant. Results are expressed as mean value ± SD.

Results

Patient characteristics (Tables 1 and 2). Baseline characteristics, left ventricular function and angiographic severity of disease were similar in the two groups, except that patients receiving dalteparin sodium were on average 6 years older than those in the placebo group.

Treadmill exercise testing (Table 2, Fig. 1 to 3). Eight (80%) of the 10 dalteparin sodium–treated and 4 (31%) of the 13 placebo-treated patients had an increase in rate-pressure product at 1-mm ST segment depression (p = 0.019) (Fig. 1). The responses were heterogeneous in both groups, such that there was no significant change in the mean magnitude of increase in rate-pressure product at 1-mm ST segment depression either in the low molecular weight heparin– or placebo-treated groups (Table 2, Fig. 2).

All low molecular weight heparin–treated patients increased the duration of exercise to the development of ischemia, compared with 8 (62%) of 13 placebo-treated

<table>
<thead>
<tr>
<th>Table 2. Results of Treadmill Exercise, Ambulatory ST Segment Monitoring and Radionuclide Ventriculography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Group</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td><strong>Treadmill exercise</strong></td>
</tr>
<tr>
<td>Rest RPP (beats/min-mm Hg·10⁻¹)</td>
</tr>
<tr>
<td>RPP at 1 mm ST depression</td>
</tr>
<tr>
<td>Duration to 1 mm ST depression (min)</td>
</tr>
<tr>
<td>Patients with chest pain</td>
</tr>
<tr>
<td>RPP at chest pain (beats/min-mm Hg·10⁻¹)</td>
</tr>
<tr>
<td>Duration to chest pain (min)</td>
</tr>
<tr>
<td>RPP at end test (beats/min-mm Hg·10⁻¹)</td>
</tr>
<tr>
<td>Duration at end test (min)</td>
</tr>
<tr>
<td><strong>Ambulatory ST segment monitoring</strong></td>
</tr>
<tr>
<td>Mean number of episodes/48 h</td>
</tr>
<tr>
<td>Mean duration of episodes/48 h (min)</td>
</tr>
<tr>
<td><strong>Radionuclide ventriculography</strong></td>
</tr>
<tr>
<td>Rest LV ejection fraction (%)</td>
</tr>
<tr>
<td>Exercise LV ejection fraction (%)</td>
</tr>
<tr>
<td>Change in LV ejection fraction (%)</td>
</tr>
<tr>
<td>Exercise heart rate (beats/min)</td>
</tr>
</tbody>
</table>

*p < 0.01, *p < 0.05. Values are expressed as mean value ± SD or number (%) of patients. LV = left ventricular; RPP = rate-pressure product.
Angiogenic Effects of Low Molecular Weight Heparin

Figure 1. Percent of patients in the dalteparin sodium (black bars) and placebo (gray bars) groups who demonstrated improvement after 1 month of therapy in 1) rate-pressure product (RPP) at the onset of 1 mm of ST segment depression during treadmill exercise, 2) duration of exercise to the onset of 1 mm of ST segment depression, 3) number, and 4) duration of episodes of ST segment depression during ambulatory monitoring, and 5) change in left ventricular ejection fraction (LVEF) with exercise. Multivariate analysis of variance using these variables demonstrated a significant difference in the dalteparin sodium group compared with the placebo group (p < 0.014). *p < 0.03.

Patients (p = 0.023) (Fig. 1 and 3). The magnitude of increase in duration of exercise was 2 ± 1.5 min (36%, p < 0.003) in the treated group and 1.5 ± 1.8 min (23%, p < 0.03) in the placebo group (Table 2).

Five (83%) of the six treated patients and three (38%) of the eight placebo group had an increase in the rate-pressure product at the onset of chest pain (p = NS). The duration of exercise to chest pain increased by 42% in the treated patients (p = 0.01), but the change was insignificant in the placebo group (Table 2).

The rate-pressure product at peak exercise increased in 80% of the dalteparin sodium–treated patients and in 69% of the placebo-treated patients (p = NS). There was a greater increase in the duration to peak exercise in the dalteparin sodium–treated patients than in the placebo group (44% vs. 20%, respectively, p < 0.03) (Table 2).

Ambulatory ST segment monitoring (Table 2, Fig. 4). Baseline. Nine of the 10 low molecular weight heparin–treated patients and 11 of the 13 placebo-treated patients had one or more episodes of ST segment depression during 48-h ambulatory ST segment monitoring. Thirty episodes (70% silent) lasting 1,064 min were recorded in the dalteparin

Figure 2. Rate-pressure product at the onset of 1 mm of ST segment depression during exercise in the dalteparin sodium (Fragmin) and placebo groups at baseline and at 4 weeks. BPM = beats per minute.

Figure 3. Duration of exercise at the onset of 1 mm of ST segment depression (STD) in the dalteparin sodium and placebo groups at baseline and at 4 weeks.

Figure 4. Duration of episodes of ST segment depression recorded during ambulatory ST segment monitoring in the dalteparin sodium (Fragmin) and placebo groups at baseline and at 4 weeks.
sodium group and 69 episodes (85% silent) lasting 2,217 min were recorded in the placebo group (p = NS).

After treatment. The duration of episodes of ST segment depression was 35% lower in low molecular weight heparin-treated than in placebo-treated patients after 4 weeks (p < 0.05), with eight of nine patients developing less ischemia (Fig. 1 and 4). In contrast, the duration of ischemia was not significantly changed (40% increase) in the placebo group, with only 4 (36%) of 11 patients experiencing less ischemia. Although the number of episodes was also reduced by 30% in the treated group, in contrast to a 2% increase in the placebo group, this difference did not achieve statistical significance (Table 2).

Radionuclide ventriculography (Table 2). There was no change in left ventricular ejection fraction at rest during the study period in either group. A decrease (2.5 ± 4.2%) in left ventricular ejection fraction with exercise at baseline was converted to a mean increase after 4 weeks of dalteparin sodium therapy (p = 0.09). The change in ejection fraction with exercise in the placebo group was not significant (Table 2). Eight (80%) of the 10 treated patients compared with 7 (54%) of 13 in the placebo group had less left ventricular dysfunction with exercise (p = 0.06) (Fig. 1). The peak exercise heart rate was not significantly different before and after 1 month of therapy (Table 2).

Multivariate analysis of variance (Fig. 1). A multivariate nonparametric analysis of variance was performed using five variables that were selected in advance because they are important determinants of collateral function (Fig. 1). Thus, when the change in the rate-pressure product and duration of exercise at 1 mm of ST depression, in the number and duration of ischemic episodes during ambulatory monitoring and in left ventricular ejection fraction with exercise after 1 month therapy were compared, there was a significant improvement in the low molecular weight heparin-treated compared with placebo-treated patients (p = 0.014).

Discussion

Rationale for use of heparin compounds. Both acidic and basic fibroblast growth factors stimulate endothelial and smooth muscle cell proliferation and migration in vitro, two processes that are central to angiogenesis (9-11,26,27). Heparin potentiates the proliferative effects of these growth factors on endothelial cells and has been found to enhance collateral function in canine models of myocardial ischemia (12,13). Of note, it has recently been demonstrated that recombinant basic fibroblast growth factor enhances collateral function by >40% in dogs (28).

Low molecular weight heparin. Dalteparin sodium (Fragmin), a fragment of heparin with a molecular weight of approximately 5,000 daltons, has several potential advantages over heparin (16-22). 1) It has 90% bioavailability after subcutaneous injection, which therefore avoids intravenous administration. 2) Its 4-h half-life is considerably longer than that of conventional heparin. 3) It has minimal antithrombin activity and its anticoagulant effects are attributable largely to its anti-Xa activity. 4) In postoperative patients, dalteparin sodium causes less bleeding than does conventional heparin. With these potential benefits over conventional heparin, the question arose as to whether a similar potentiation of collateral function could be achieved with low molecular weight heparin fragments as had been achieved with heparin.

The present investigation is the first double-blind, placebo-controlled study assessing the effects of ischemia and heparin compounds in patients with stable coronary artery disease. The study was designed as a pilot investigation to determine the feasibility of conducting such a study and to help design a large scale study to assess efficacy. We studied several indirect measures of collateral function. The rate-pressure product at the onset of ischemia (1-mm ST segment depression) was used as evidence of the maximal capacity of the coronary circulation to supply oxygen to the myocardium. Other less specific indexes of collateral function that were measured included the duration of exercise to ischemia, magnitude of ischemia during daily living (using ambulatory ST segment monitoring) and the degree of left ventricular dysfunction with exercise.

Although the results cannot be considered conclusive, the data suggest a beneficial effect of low molecular weight heparin on collateral function (Fig. 1). Compared with the placebo group, significantly more patients who received the low molecular weight heparin had improved combined indexes of collateral function. Thus, in the 80% of the treated patients who demonstrated less exercise-induced ischemia, there was an 8.5% increase in the rate-pressure product and a 54% increase in exercise duration to ischemia. Additionally, there was a 35% reduction in the ischemia experienced during daily life and an improvement of left ventricular dysfunction induced by exercise in 80% of patients treated with the low molecular weight heparin (Fig. 1). When all five factors reflecting collateral function were considered together in a multivariate analysis of variance, there was a significant improvement in low molecular weight heparin-treated compared with placebo-treated patients (p = 0.014).

These results are in concordance with a nonrandomized study (14) using heparin and exercise; the magnitude of improvement in the rate-pressure product we observed was similar to that reported in that study, which also demonstrated an increased angiographic collateral score. The improved collateral function implied by our findings could be due to either an increase in collateral growth or an opening of preformed collateral vessels. Although an improvement in these exercise indexes is in keeping with improvement in collateral function, it may also be due to other as yet unknown effects of low molecular weight heparin on myocardial oxygen demand or to its thrombolytic properties, which may lead to improvement in coronary blood flow during exercise by reducing lesion severity.

Limitations of the study. Patients with coronary artery disease often experience a training effect with regular exer-
cise, and the possibility must always be considered that collateral function may be stimulated by exercise alone (29-35). In fact, the placebo group demonstrated a significant training effect as evidenced by an increase in the duration of exercise to ST segment depression and to peak exercise; however, there was no increase in the rate-pressure product at these times.

The severity of the stenosis, the patency of the feeder vessels that are potential sources of collateral development and the magnitude and duration of the ischemic stimulus are all among the more important determinants of collateral development (6,8,36). Thus, significant collateral growth is unlikely to occur in vessels that are <80% narrowed, if there is significant narrowing in all feeder vessels, or if the duration of ischemia is limited. To avoid these potential confounding effects, we 1) selected patients with objective evidence of myocardial ischemia so that there would be a good potential for collateral growth, 2) randomized patients with three-vessel disease and previous coronary bypass surgery separately because these groups may have had a variable and perhaps reduced potential for collateral development compared with that of other patients, and 3) attempted to standardize the ischemic stimulus during the first two weeks of treatment by exercising patients to ischemia three times a day. Despite these measures, it is clear that not all patients in the low molecular weight heparin group responded similarly. Because of the small number of patients studied, stratification into subgroups cannot be performed and our results therefore need to be confirmed by a larger randomized study.

The conclusion that an increase in rate-pressure product at the onset of 1 mm of ST depression during exercise indicates improved collateral function is based on the assumptions that the rate-pressure product is an indirect measure of myocardial oxygen demand and that collateral flow is maximal at the development of ischemia. However, other determinants of myocardial oxygen demand, such as wall stress and contractility, were not measured. Nevertheless, the higher rate-pressure product reached in 80% of dalteparin sodium-treated patients after a longer period of exercise time than that in the placebo group makes it unlikely that this higher rate-pressure product was being offset by a simultaneous reduction in contractility and wall stress.

Conclusions. The findings from this pilot study provide preliminary evidence suggesting that exercise and low molecular weight heparin therapy with dalteparin sodium decrease myocardial ischemia in patients with stable coronary artery disease and that the decrease is likely to be mediated by enhanced collateral function. Whether a longer duration of therapy, a higher dose of drug or more exercise training would have achieved an increased angiogenic response and whether the observed improvement will persist over time need to be investigated further in a long-term trial.


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