Benefit of Adding Low Molecular Weight Heparin to the Conventional Treatment of Stable Angina Pectoris

A Double-Blind, Randomized, Placebo-Controlled Trial

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Background. Patients with chronic coronary artery disease exhibit a dysfunctioning endothelium, which may be responsible for exercise-induced platelet activation and expression of a procoagulant moiety. In this study, we evaluated the therapeutic efficacy of a low molecular weight heparin (Parnaparin) in patients with stable angina pectoris.

Methods and Results. According to a double-blind, randomized, placebo-controlled trial, 29 patients with stable exercise-induced angina pectoris and angiographically proven coronary artery disease received a single daily subcutaneous injection of Parnaparin or placebo on top of aspirin and conventional antiangiinal medication over 3 months. Patients randomized to Parnaparin showed a significant decrease in the fibrinogen level (P=0.035) and an improvement in both the time to 1-mm ST segment depression (P=0.006) and the peak ST segment depression (P=0.015). The Canadian Cardiovascular Society class for angina pectoris was also improved by Parnaparin (P=0.016). Parnaparin did not affect ADP and collagen-induced platelet aggregation, whereas thrombin-induced aggregation was reduced (P=0.001). The bleeding time was slightly prolonged, but this was not associated with any significant bleeding.

Conclusions. Patients with stable angina pectoris may be treated with Parnaparin in addition to aspirin and conventional antiangiinal medication. Side effects are negligible, and compliance is excellent. (Circulation. 1993;88:2517-2523.)

KEY WORDS • angina • heparin • fibrinogen

Although coronary atherosclerosis is the common process underlying virtually all the clinical manifestations of ischemic heart disease, a clear distinction between acute coronary syndromes and chronic, stable coronary disease is traditionally made. Plaque disruption with different degrees of thrombus formation is known to be responsible for most cases of instability.1 As a consequence, antithrombotic therapy with antiplatelet, anticoagulant, and fibrinolytic agents is firmly established in these situations. In patients with stable coronary artery disease, angina pectoris results from an increase in myocardial oxygen consumption with different degrees of superimposed vasoconstriction.2 Accordingly, combination therapy with β-blockers, nitrates, and calcium blockers has gained popularity. However, coronary atherosclerosis is a chronic process of continuous remodeling of the arterial tree due to the dynamic occurrence of types I-II and III vascular injury.3 Even in the presence of mild vascular injury, the potential exists for platelets to become attached and blood coagulation to be initiated. Recently, it has been shown in patients with stable coronary artery disease that platelet activation takes place across the coronary bed in response to rapid atrial pacing, provided that >50% narrowing in one or more branches is present.4 In an autopsy study of patients with coronary artery disease who died of noncardiac causes, Davies et al5 found that 17% of patients had fissures in atherosclerotic plaques, and in some cases, overlying thrombi were found as well. Platelet adhesion and aggregation in turn lead to the release of a host of vasoactive substances such as thrombin, serotonin, thromboxane A2, ADP, and platelet-activating factor, which exert a vasoconstrictive effect either through the enhancement of endothelium-derived contracting factors or directly.6-9 Thus, a “continuum” exists between stable and unstable coronary disease, in terms of both vascular injury and clinical manifestations.

On the basis of these arguments, we designed a trial to investigate whether myocardial ischemia can be improved after long-term treatment with low molecular weight heparin (Parnaparin) in patients with stable coronary artery disease and reproducible effort-induced myocardial ischemia.

Methods

Patients

Patients aged 40 to 79 years who had experienced anginal symptoms of a stable pattern for a minimum of
3 months and were not currently evaluated for coronary artery bypass graft surgery or percutaneous transluminal coronary angioplasty were considered for the study. All patients had angiographically proven coronary artery disease, and objective demonstration of ischemia during exercise testing was mandatory. The exclusion criteria comprise the presence of any clinically important concomitant disease (in particular, myocardial infarction within the previous 3 months; renal impairment, described as serum creatinine >2.0 mg/dL; hepatic function impairment, defined as aspartate transaminase or alanine transaminase enzyme results >15% above the upper normal limit and deemed to be clinically significant; anemia, defined as a hemoglobin concentration of <12 g/dL; hypotension, defined as standing systolic blood pressure <100 mm Hg; and hypertension, defined as systolic blood pressure >200 mm Hg or diastolic blood pressure >110 mm Hg), contraindications to the use of anticoagulants (history of previous significant bleeding or malignancy), and/or the presence of confounding factors for the interpretation of the ECG (patients with left ventricular hypertrophy and resting ST-T wave abnormalities on the ECG, predominant cardiac rhythm other than sinus rhythm, concurrent treatment with digoxin). All patients were treated with β- and calcium blockers in association with nitrates and aspirin (325 mg/d).

Study Protocol

Baseline measurements were performed during a run-in period of 2 weeks, during which all patients underwent two diagnostic treadmill exercise tests on full conventional medical treatment (as outlined above) to show reproducible ischemia (<10% difference in the ergometric data). Thereafter, patients were randomized in a double-blind, parallel-group comparison to receive a 3-month treatment period of a single daily subcutaneous injection of either Parnaparin (6400 U according to the first World Health Organization International Standard for low molecular weight heparins) or matching placebo. Parnaparin, also variously identified as OP/2123, Alfa-LMWH, Fluxum (Alfa-Wassermann, SpA, Bologna, Italy), is obtained by degradation of crude porcine mucosal heparin in presence of cupric acetate and H₂O₂, followed by ethanol extraction. This agent has an average molecular weight of 5000 da and specific activity of 85 U/mg in antifactor-Xa assay and 35 U/mg in the activated partial thromboplastin time (aPTT) assay. The ratio between both types of activities in tests using human plasma is 2.4. Previous work from our and other groups has shown that Parnaparin results in significant anti-Xa activity, aPTT levels, and in a decrease in fibrinopeptide A concentration for at least 12 hours and often for 24 hours after a single subcutaneous injection in patients with acute myocardial infarction and healthy subjects. Treadmill exercise testing was repeated at the end of the 3-month treatment period, and the angina pectoris class was monitored according to the Canadian Cardiovascular Society.

Exercise Testing

Exercise testing was performed with the modified Bruce protocol on a computerized treadmill system (Marquette case 12). Twelve-lead ECG recording and blood pressure measurement (cuff) were taken at rest, at the end of each minute during exercise, at the point of 1 mm (0.1 mV) ST segment depression, at peak exercise, and 3 and 6 minutes into the recovery period. Three ECG leads were monitored continuously before and during exercise and for 10 minutes into recovery. All tests were performed at the same time of day, 4 hours after the last dose of treatment for each patient. A positive exercise test diagnostic of myocardial ischemia was defined as horizontal or downsloping ST segment depression >1 mm measured 80 milliseconds after the J point with respect to the resting value. The exercise test was stopped in the event of chest pain of moderate severity or inability of the patient to exercise further. For each exercise test, the level of the ST segment was calculated after signal averaging by a computer-assisted system in all 12 leads every minute with an accuracy of 0.1 mm. The lead showing the greatest ST segment depression was selected for analysis. The values then measured were exercise time (minutes), amount of ST segment depression at peak exercise (mm), and the heart rate–blood pressure product (beats per minute×mm Hg) at the start of 1-mm ST segment depression and at peak exercise. The exercise tests were analyzed by investigators blinded to the treatment. The results of the two baseline examinations were averaged for the purpose of analysis.

Hemostasis Analyses and Biochemistry

Venous blood was collected by clean venipuncture through an 18-gauge needle and without tourniquet, if possible. The first 2 mL were discarded. Anti-Xa activity was assayed by blood collected in tubes containing sodium citrate 3.8%, according to the antifactor-Xa assay of Teien et al. and Teien and Li (Coatest Kabivitrum; Stockholm, Sweden). The aPTT was determined automatically on a MLA Electra 1000 C coagulometer using as reagent Actin FS (Dade; supplied by Baxter, Milan, Italy). The fibrinopeptide A concentration was determined after nine parts of whole blood were mixed in a plastic tube with one part of anticoagulant containing in each milliliter 32 mg Tris-citrate, 1000 IU heparin, and 1 TIU aprotinin. Samples were centrifuged within 10 minutes at 3000 rpm for 10 minutes; plasma was stored at −30° after being depleted of fibrinogen by bentonite absorption. Fibrinopeptide A levels were measured by an enzyme-immunoassay within 1 month (Stago; supplied by Boeringer Biochemistry, Milan, Italy). Blood samples were drawn before treatment allocation (baseline) and 4 hours after the first injection of either Parnaparin or placebo and after 1 to 2 and 3 months (always 4 hours after the injection). Fibrinogen (coagulation rate assay of Clauss) and the principal biochemical data were measured at the baseline and after 3 months.

Platelet Aggregation

Blood for ex vivo platelet aggregation was collected into a 10-mL syringe and mixed with sodium citrate solution (3.8%) and diluted 1:1 with 0.9% NaCl. Samples were obtained before the first dose, 4 hours after the first dose, and after 3 months (4 hours after the last injection). All aggregations were performed in a dual-channel aggregometer (Chrono-log Corporation; supplied by Mascia-Brunelli, Milan, Italy) at 37°C with constant stirring of diluted whole blood at 1000 rpm.
Quantitative analysis of aggregation was determined as the maximum change in electrical impedance ($\Omega$) after addition of agonist. As aggregating agents, we used ADP (10 and 20 $\mu$mol), collagen (2 and 5 $\mu$g), and human $\alpha$-thrombin (0.1 U/mL).

**Bleeding and Template Bleeding Time**

The template bleeding time was measured before, after the first injection, and after 1 to 2 and 3 months, always 4 hours after the scheduled injection. A spring-activated surgical-steel blade instrument (Surgicut International, Technidyne Corp) designed to produce a standardized incision (5 mm long by 1 mm deep) was applied parallel to and below the antecubital crease after a cuff sphygmomanometer was inflated up to 40 mm Hg. Flow of blood from the wound was blotted at 30-second intervals with filter paper disks, carefully avoiding direct contact with the wound. The duration of bleeding was quantitated by timing the interval (to the nearest 30 seconds) from incision to cessation of bleeding as demonstrated by no further staining of the filter paper. The occurrence of spontaneous bleeding was closely monitored for the duration of the trial.

**Statistical Analysis**

Baseline characteristics in the two treatment groups were compared by the unpaired $t$ test (continuous data) and the $\chi^2$ test (categorical data). The control and posttreatment data were compared by either the paired $t$ test or ANOVA. When the $F$ value was statistically significant by ANOVA, multiple comparisons were made by the Newman-Keuls test. Changes in ergometric data and in fibrinogen level were submitted to linear regression analysis with computation of the coefficient of correlation. A two-tailed $P$ of <.05 was considered statistically significant. Data are given as mean±SD.

**Results**

A total of 29 patients were included in the trial, of whom 15 were allocated to Parnaparin therapy and 14 were assigned to placebo. Baseline characteristics in the two groups were well balanced (Table 1), particularly with regard to the extent of coronary atherosclerosis, the impingement of coronary risk factors, and the use of concomitant, conventional medication. Parnaparin injection resulted in anti-Xa levels of 0.37±0.24 U/mL after 4 hours. There was no accumulation of the drug over the 3-month treatment period (Table 2). Likewise, the aPTT increased from 24±4 to 36±9 seconds 4 hours after Parnaparin injection ($P<.0001$) and subsequently did not increase further. During Parnaparin therapy, fibrinogen levels were reduced from 387±90 to 333±65 mg/dL after 3 months ($P<.035$; Fig 1), whereas it was unchanged in the placebo group (from 346±60 to 345±56 mg/dL; $P=NS$). Baseline fibrinopeptide A levels were low in both groups, and low levels were found throughout the course of the study.

In the Parnaparin group, compared with the control, the exercise time to 1-mm ST segment depression increased from 285±126 to 345±168 seconds ($P=.008$; Table 3). The increment was also associated with an increase in the rate-pressure product at 1-mm ST segment depression from 17.310±4030 to 18.890±4740 ($P=.07$). In this respect, the changes observed in the placebo group were not statistically significant. The time to peak exercise was slightly prolonged in both the Parnaparin and the placebo group, and the peak rate-pressure product was unchanged in the two groups. However, the peak ST segment depression decreased from 2.13±0.51 to 1.91±0.62 mm in the Parnaparin group ($P=.015$), whereas it was unchanged in the placebo group (2.08±0.9 versus 2.12±0.8 mm; $P=NS$; Fig 2). The time to the onset of moderate angina pectoris during exercise was slightly reduced over 3 months in the placebo group (from 284±122 to 268±80 seconds), whereas it was mildly prolonged in the Parnaparin group (from 246±83 to 277±112 seconds); however, the difference was not statistically significant. The Canadian Cardiovascular Society class for angina pectoris was statistically improved in the Parnaparin group (from 2.27±0.75 to 1.83±0.77 at 1 month, 1.87±0.74 at 2 months, and 1.90±0.69 at 3 months; $P=.016$ by ANOVA) but was unchanged in the placebo group (2.21±0.93 versus 2.21±1.12, 2.46±1.08, and 2.36±1.17; Fig 3). Neither the improvement in exercise duration at 1-mm ST depression nor the change in peak ST depression at peak exercise was significantly related to the changes in fibrinogen level ($r=−.117$ and .105, respectively; both $P=NS$; Fig 4).

Table 4 summarizes the results of ex vivo platelet aggregation. No substantial variations in the extent of ADP and collagen-induced aggregation from baseline measurements were detected during Parnaparin therapy. Interestingly, the drug was able to decrease thrombin-induced aggregation (from 17.9±10.8 to 4.5±10.1 $\Omega$).
TABLE 2. Hemostatic Variables

<table>
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<tr>
<th></th>
<th>Baseline</th>
<th>4 Hours</th>
<th>1 Month</th>
<th>2 Months</th>
<th>3 Months</th>
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<tr>
<td>Anti-Xa, U/ml</td>
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<td>Parnaparin*</td>
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<td>0.37 (0.24)</td>
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<td>0.42 (0.23)</td>
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<td></td>
<td>...</td>
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<td>...</td>
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<td>aPTT, s</td>
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<td>Parnaparin*</td>
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<td>36 (9)</td>
<td>32 (5)</td>
<td>33 (6)</td>
<td>34 (5)</td>
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<tr>
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<td>24 (3)</td>
<td>25 (3)</td>
<td>24 (4)</td>
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<tr>
<td>Fibrinopeptide A, ng/mL</td>
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<td></td>
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<td>Parnaparin</td>
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<td>4 (8)</td>
<td>1 (1)</td>
<td>4 (7)</td>
<td>3 (4)</td>
</tr>
<tr>
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<td>4 (5)</td>
<td>4 (6)</td>
<td>5 (6)</td>
<td>3 (2)</td>
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<tr>
<td>Bleeding time, s</td>
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<tr>
<td>Parnaparin</td>
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<td>532 (181)</td>
<td>544 (227)</td>
<td>436 (172)</td>
<td>484 (172)</td>
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<tr>
<td>Placebo</td>
<td>425 (151)</td>
<td>372 (98)</td>
<td>388 (130)</td>
<td>378 (106)</td>
<td>413 (139)</td>
</tr>
</tbody>
</table>

Standard deviations are given in parentheses.

Data at 1 to 2 and 3 months were collected 4 hours after the injection of Parnaparin (or placebo).

*P<.001 by ANOVA (all the variability being explained by the difference between baseline and the following data).

†P<.007 by ANOVA (all the variability being explained by the difference between baseline and data at 4 hours and 1 month).

Discussion

The goal of therapy of stable angina pectoris is to abolish or reduce anginal attacks and myocardial ischemia and to promote a more normal lifestyle. To this end, a number of pharmacologic and revascularization tools are presently available either to lower the myocardial oxygen demand or to increase the coronary blood flow. However, myocardial ischemia is frequently present notwithstanding optimal medical therapy including full doses of β-blockers and calcium blockers plus nitrates. Some relief is afforded by new interventional catheterization procedures, and a more definite control of symptoms is observed after coronary bypass surgery. However, disease progression after revascularization as well as diffuse disease, especially in the presence of concomitant disorders such as diabetes mellitus and hyperlipidemia, significantly increase the total ischemic burden.

Patients with coronary artery disease exhibit a dysfunctioning endothelium in the form of paradoxical vessel narrowing during acetylcholine challenge or in face of increased myocardial oxygen demand. A dysfunctioning endothelium may also express a procoagulant moiety whereby the interaction of vessel wall with...
circulating blood cells and coagulation factors is actually facilitated, particularly at high shear-rate conditions. Recent studies have shown that in advanced and fibrous plaques, products related to platelets and fibrin may be detected in the intima, the neointima, and even in the deeper medial layer. Any local accumulation of agents like thromboxane A₂, serotonin, ADP, ATP, and thrombin may in turn further increase the vascular tone thus precipitating myocardial ischemia.

With the above considerations in mind, we decided to test the hypothesis that chronic treatment with a low molecular weight heparin, Parnaparin, would confer some benefit to patients with stable angina pectoris already taking conventional antianginal medications in addition to aspirin. Heparin is at least as effective as aspirin in the management of unstable angina. However, because the same may not be true in the chronic phase of the disease in which aspirin has been shown to prevent new vascular events, we decided that any possible benefit deriving from Parnaparin should be demonstrated on top of aspirin. We chose a low molecular weight heparin because the prolonged plasma half-life and the excellent bioavailability of these agents offer the convenience of single daily administration. Parnaparin was previously investigated by our and other groups and has been shown to result in significant anti-Xa activity and aPTT levels over 24 hours after a single subcutaneous injection. Due to the lack of interference by platelet factor 4, the drug also is more active than unfractionated heparin in plasma rich in activated platelets, which is usually the case in the proximity of tight coronary stenosis. A large experience with low molecular weight heparins, including

![Bar graph of peak ST depression before and after treatment with Parnaparin or placebo.](image)

![Bar graph of Canadian Cardiovascular Society functional class for angina pectoris in the two treatment groups.](image)
use as adjunctive therapy during coronary thrombolysis with recombinant tissue-type plasminogen activator. Our study shows that a 3-month course of Parnaparin is able to decrease the fibrinogen level in patients with stable coronary artery disease. The data are in keeping with those observed in patients with peripheral arterial disease and are important because fibrinogen is a well known systemic risk factor for thrombosis and ischemic heart disease. Fibrinogen is one of the principal determinants of plasma and whole-blood viscosity, which are known to be increased in patients with stable angina pectoris. Furthermore, a positive correlation exists between red cell aggregation and fibrinogen concentration, suggesting that altered blood rheology and blood flow may be of importance in the development of myocardial ischemia and vascular occlusion. Likewise, platelet deposition and residence time as well as the convective transport of relevant solutes (ie, oxygen, ADP, thrombin, other plasma components) near a region of disturbed flow are influenced by the rheology of blood. In addition, fibrinogen levels within the physiological range increase platelet aggregability and the amount of fibrin formed when the coagulation is initiated. Another finding of this study is that Parnaparin reduces thrombin-induced platelet aggregability. Thrombin is responsible for a host of unfavorable effects, including proteolytic activation of factors II, V, VIII, and XIII; platelet aggregation; vasoconstriction; neutrophil adherence; monocyte chemotaxis; mitogenesis for smooth muscle cells and lymphocytes; and stimulation of production of platelet-activating factor and platelet-derived growth factor. Therefore, it is conceivable that Parnaparin interference with thrombin-induced platelet aggregation may result in reduced coronary vasoconstriction and myocardial ischemia. Theoretically, one might hypothesize that blood delivery to the compromised myocardium through collateral channels is augmented by Parnaparin because heparin-binding growth factors such as acidic fibroblast growth factor (also referred to as aFGF or HBGF-1) and basic fibroblast growth factor (bFGF or HBGF-2) directly stimulate proliferation and migration of endothelial cells and fibroblasts, resulting in new vessel growth. In this context, it is interesting to see that fibrin may enhance collateral growth in dogs subjected to repeated brief coronary occlusion and the development of functional anastomotic channels between an implanted internal mammary artery and a collateral-dependent, ameori-obstructed left anterior descending coronary artery.

The improvement in exercise tolerance that we observed was quantitatively similar to that generally documented with the use of classic antiangiinal drugs and, importantly, was paralleled by a subjective improvement in symptoms, as reflected in the lower class for angina pectoris. In our view, these are important clinical end points and deserve some consideration, particularly because they have been obtained with the additional use of other medications. Patient compliance was excellent since the treatment was not withheld in any case and no side effects were reported, except for a small, single subcutaneous hematoma in a patient over the 3-month course of therapy.

We conclude that an antithrombotic treatment with low molecular weight heparin may improve the fibrinogen level, the exercise tolerance, and symptoms of angina pectoris in patients with stable coronary artery disease. Thus, a new therapeutic mechanism may be available for the growing number of patients still having myocardial ischemia on conventional optimal treatment. Further advantages, in terms of recurrent hospitalization, need of mechanical revascularization, and occurrence of myocardial infarction, may be addressed by large-scale clinical trials that are currently ongoing.

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