Effects of Various Anticoagulant Treatments on von Willebrand Factor Release in Unstable Angina

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OBJECTIVES

We tested the hypothesis that different anticoagulant treatments may produce different platelet effects and von Willebrand factor (vWf) release in unstable angina.

BACKGROUND

The early increase of vWf has been reported to be a risk factor for adverse outcome in unstable angina. Anticoagulant drugs play a key role in stabilization of unstable angina, but they may not have the same efficacy and the same effects on acute vWf release.

METHODS

We studied 154 patients enrolled in several clinical trials testing four different anticoagulant treatments in unstable angina or non-Q-wave myocardial infarction. Patients were treated during at least 48 h by either intravenous unfractionated heparin, one of two different low molecular weight heparins (enoxaparin or dalteparin) or the direct thrombin inhibitor PEG-hirudin. All patients received aspirin but no IIb/IIIa inhibitors.

RESULTS

The release of vWf over the first 48 h (Δ vWf) did not relate to the baseline clinical characteristics. At 30 days of follow-up, Δ vWf was sevenfold higher in patients with an end point (death, myocardial infarction, revascularization) than in patients free of events (+53 ± 7% vs. +7 ± 14%, p = 0.004). The same trend was present for each component of the composite end point with the highest levels for one-month mortality (+87 ± 32% vs. +26 ± 8%, p = 0.09). The vWf values did not increase over 48 h in patients receiving either enoxaparin or PEG-hirudin (+10 ± 9% and −5 ± 20%, respectively). A serious rise of vWf was measured in unfractionated heparin-treated patients (+87 ± 11%), which differed significantly from the enoxaparin group (p = 0.0006) and PEG-hirudin group (p < 0.001). In dalteparin-treated patients, Δ vWf was elevated (+48 ± 8%) and did not differ from the unfractionated heparin group (NS).

CONCLUSIONS

We confirm that, in unstable angina patients, a rise of vWf over the first 48 h is associated with an impaired outcome at 30 days. Moreover, the four different anticoagulant treatments tested here do not provide the same protection with regards to vWf release, which may have important prognostic implications and explain different results observed in recent clinical trials. (J Am Coll Cardiol 2000;36:110–4) © 2000 by the American College of Cardiology

In recent years, the management of patients with unstable angina has greatly improved in both risk stratification and drug therapy. Few biochemical markers bring additive prognostic information to what is known from medical history, symptoms, and electrocardiogram (ECG); troponins, C-reactive protein, fibrinogen or von Willebrand factor (vWf) can participate to a better risk stratification of patients. We recently reported the predictive value for adverse outcome at one-month of an early rise of vWf in unstable angina patients recruited in a substudy of the ESSENCE trial (1). Furthermore, a treatment effect was present with less release of vWf on enoxaparin than on unfractionated heparin treatment, but several questions remained unanswered. We did not know whether it was a specific property of enoxaparin or a low molecular weight heparin class effect, or even a common characteristic of anticoagulant treatments clinically more effective than unfractionated heparin in unstable angina.

To date, only two anticoagulant drugs have shown superior results to unfractionated heparin in unstable angina: the low molecular weight heparin enoxaparin in both ESSENCE and TIMI-11B (Thrombolysis in Myocardial Infarction) trials and, the direct thrombin inhibitor lepirudin in OASIS 1 and 2 trials (2–5). Dalteparin, a different low molecular weight heparin, has shown equivalent results to unfractionated heparin (6). The increase of vWf being a predictor of clinical outcome in unstable angina, we hypothesized that its control may relate to the efficacy of anticoagulation and differ between the various anticoagulant treatments evaluated in unstable angina. We decided to measure vWf release over the first 48 h of treatment in unstable angina patients receiving four different anticoagulant therapies: continuous IV unfractionated heparin adjusted to activated partial thromboplastin time (aPTT), enoxaparin given subcutaneously twice daily, dalteparin...
given subcutaneously twice daily, and continuous IV. PEG-hirudin, a direct thrombin inhibitor with a favorable pharmacologic profile, tested successfully in a phase II trial (7).

The vWF, a multimeric protein of the acute-phase reaction, is stored in the Weibel-Palade bodies of endothelial cells and in the platelet alpha-granules and can be released rapidly at the local site of the injured artery; vWF is crucial for both platelet adhesion to exposed subendothelium and platelet aggregation. Recently, in the plasma of patients with acute myocardial infarction, increased vWF plasma concentrations was the main determinant for the enhancement of platelet aggregation at high shear rates (8). The vWF plasma levels, heightening shear-induced aggregation, may have a causative role in acute coronary thrombotic events. Furthermore, epidemiological studies have shown vWF to be a risk factor for coronary heart disease, and elevated levels have been measured in acute coronary situations like myocardial infarction, unstable angina and coronary angioplasty (1,9–12). Release of vWF in unstable angina reflects the seriousness of the platelet-mediated event, and its relation to anticoagulant treatment is a real issue for this new marker of prognosis (1).

METHODS

Study population. We studied 154 patients with unstable coronary artery disease defined as rest angina lasting more than 10 min, with the last episode of chest pain within the last 24 h, and clear evidence of coronary artery disease as shown by at least one of the following: 1) ECG changes; 2) a rise in creatine kinase (CK) and/or troponin I; 3) previous myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass graft (CABG); 4) previous positive exercise test; and 5) previous coronary angiography showing a significant stenosis of any coronary artery.

Exclusion criteria included persistent ST-segment elevation, contraindications to anticoagulation, current need for oral anticoagulant (e.g., heart valve prosthesis), and severe renal failure.

The study patients were enrolled in different centers participating in trials (TIMI-11B, ESSENCE, USIC-registry, PEGHIRUD 022) that evaluated low molecular weight heparins, PEG-hirudin and unfractionated heparin in unstable angina. Therefore, patients were not directly randomized among the four treatment groups, but there was no bias in the allocation to the different anticoagulant treatments. In all these trials, anticoagulation was always administered for a minimum of 48 h, and the last blood sampling was always obtained on treatment. Informed consent was obtained from all patients, and the study was approved by the Human Research Committee of the Institutional Review Board.

Study design. All patients were treated with aspirin (100 to 325 mg daily), beta-blockers and IV nitrates unless contraindicated. None of the patients received IIb/IIIa inhibitors. Anticoagulation was performed with either unfractionated heparin, enoxaparin, dalteparin, or PEG-hirudin. Patients treated with unfractionated heparin received an IV bolus (5,000 IU) followed by a continuous infusion at a dose adjusted to the activated partial-thromboplastin time, targeting values between 1.5 and 3.0 times control (according to the study). Enoxaparin was given at a dose of 100 anti-Xa units/kg, subcutaneously at 12-h intervals. Dalteparin was administered at a dose of 120 anti-Xa units/kg, subcutaneously at 12-h intervals. Escalating doses of PEG-hirudin were used (phase II trial), combining a bolus dose of 0.1 mg/kg with an infusion of 0.02, 0.025, 0.03 or 0.035 mg/kg/h IV. Doses of PEG-hirudin were not adjusted to aPTT; however, for safety reasons the upper limit of aPTT was set at 3.0 times control and the infusion was stopped in case the patient exceeded the set limit. Infusion was re-started immediately after the aPTT was below 3.0 times control (aPTT being rechecked every 2 h).

End points were evaluated at 30 days, including death, new myocardial infarction or revascularization procedure. For the purpose of the present study, we used the database of each trial in which patients were enrolled, with all demographic data, baseline characteristics, validated clinical events and revascularisation procedures. We evaluated each individual end point as well as the triple end point combining death, myocardial infarction, revascularization procedures, and the double end point associating death and myocardial infarction.

Antigen measurements of vWF. Blood was sampled twice in each patient on admission (baseline) and at 48 h. Venous blood (9 volumes) was collected into a Vacutainer tube containing 0.129 mol/liter trisodium citrate (1 volume) for vWF antigen measurements. Platelet-poor plasma was obtained by centrifugation at 3000g for 20 min at 10°C. Plasma was aliquotted and stored at −80°C. Frozen samples were transported in dry ice from each center to the central laboratory, and the quality of frozen samples was checked on delivery. All measurements were performed in a blinded fashion in the central laboratory of Pitie-Salpetriere Hospital. As previously described, the plasma concentrations of vWF antigen were measured in duplicate with an enzyme-linked immunosorbent assay (ELISA) technique (Asserachrom vWF, Diagnostica Stago, Asnières, France) (12). The enzyme immunoassay procedure was controlled as recommended by the manufacturer. The normal range of the laboratory evaluated in healthy volunteers was 60% to 110%. The interassay and intraassay coefficient of variation was
Changes of vWF > 48 h (Δ vWF) were calculated as Δ vWF = vWF at H₄₈ - vWF at H₀ (baseline).

**Statistical analysis.** Results are expressed as mean ± SEM. Simple linear regression was used to test the association between continuous variables. Potential associations between clinical or biological parameters were tested by univariate procedures using the Student t or chi-square tests, with an alpha level set at 0.05. The significance of differences among different treatment groups were determined by analysis of variance (ANOVA) and post hoc t tests with Bonferroni correction, the level of significance being p = 0.0083 after correction for the multiple comparisons.

**RESULTS**

**Clinical characteristics.** We recruited 154 patients into this study. Thirty-nine patients were assigned to unfractionated heparin (69% men, mean age 68 ± 2 years), 44 to PEG-hirudin (72% men, mean age 61 ± 2 years), 40 to enoxaparin (58% men, mean age 69 ± 2 years) and 31 to dalteparin (66% men, mean age 66 ± 2 years). Thirty-four patients (22%) were more than 75 years old. The admission diagnosis was unstable angina in 113 patients (73%) and non-Q-wave myocardial infarction in 41 patients (27%). Changes in ECG were present in 125 patients (81%). Forty-three patients (28%) had a prior history of myocardial infarction, 12 patients (8%) had a prior CABG, 27 patients (18%) had a prior PTCA, and the distribution of baseline clinical characteristics was similar in the four treatment groups. Complete clinical follow-up at 30 days was obtained in 150 patients (98%). At one month, death occurred in eight patients (5%), myocardial infarction in 12 patients (8%) and non-Q-wave myocardial infarction in 17 patients (11%) within the first month.

**Release of vWF and baseline characteristics.** Mean vWF plasma level of the entire study population was 199 ± 10% at baseline and increased further to 230 ± 9% at 48 h. The vWF levels at baseline and at 48 h as well as the release over the first 48 h (Δ vWF) did not relate significantly to any of the baseline clinical characteristics. The Δ vWF did not correlate with age, but there was a trend to higher Δ vWF values in patients over 70 years (Table 1). Similarly, a slight but nonsignificant increase of Δ vWF was measured in patients with a prior history of myocardial infarction, a prior CABG, or a non-Q-wave myocardial infarction (compared to unstable angina), all characteristics usually associated with an impaired prognosis (Table 1).

**Effects of anticoagulant treatments on vWF release.** The early rise of vWF measured in unfractionated heparin-treated patients was not observed in the two groups of unstable angina patients treated with either PEG-hirudin or enoxaparin (see Fig. 1). In contrast, an early increase of vWF was present in dalteparin-treated patients, which did not differ significantly from the rise measured in the unfractionated heparin group, although of smaller magnitude.

Over the first two days, vWF increased in 83% of patients who experienced a clinical event within the 30 days of follow-up. The Δ vWF was sevenfold higher in patients with an end point (death, myocardial infarction, revascularization) at one month than in those free of end point (p = 0.004; see Fig. 2). The same trend was present for each component of the composite end point; seven patients died between 48 h and one month with Δ vWF peaking at 87 ± 32% in these eight patients (p = 0.09 vs. the rest of the population).

In each treatment group, mean Δ vWF was always higher in patients with an event compared to those free of events at one-month follow-up. Mean Δ vWF values were particularly low in patients without events treated with either PEG-hirudin or enoxaparin ( − 25 ± 34% and 0 ± 10% in PEG-hirudin and enoxaparin groups, respectively). In contrast, mean Δ vWF was 27 ± 7% in patients with a favorable...

### Table 1. Von Willebrand Factor Release and Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th></th>
<th>Δ von Willebrand Factor (%)</th>
<th>p Value</th>
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<tbody>
<tr>
<td>Males</td>
<td>25 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>42 ± 15</td>
<td></td>
</tr>
<tr>
<td>Age &lt; 70 yrs</td>
<td>16 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Age &gt; 70 yrs</td>
<td>48 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>No prior MI</td>
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<tr>
<td>Prior MI</td>
<td>47 ± 15</td>
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</tr>
<tr>
<td>No prior CABG</td>
<td>28 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Prior CABG</td>
<td>65 ± 18</td>
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</tr>
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<td>No ECG changes</td>
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<td>NS</td>
</tr>
<tr>
<td>ECG changes</td>
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</tr>
<tr>
<td>Unstable angina</td>
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<td>NS</td>
</tr>
<tr>
<td>Non-Q-wave MI</td>
<td>41 ± 16</td>
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MI: myocardial infarction; CABG: coronary artery bypass graft.

**Figure 1.** Absolute changes of von Willebrand factor levels over 48 h in patients receiving either enoxaparin, unfractionated heparin, PEG-hirudin, or dalteparin. Differences among treatment groups were analyzed by ANOVA and post hoc t tests with Bonferroni correction for multiple comparisons: after correction, comparisons were significant if p value was less than 0.0083. Δ von Willebrand factor was significantly lowered with PEG-hirudin and enoxaparin compared to unfractionated heparin.
the enhancement of platelet aggregation with heparin (16,18,20), and Xiao and Theroux (21) have shown differences of platelet stimulation between unfractionated heparin and enoxaparin in 43 unstable angina patients, but no relationship to clinical outcome was reported. We demonstrate here that ∆ vWF, a marker of both platelet stimulation and adverse clinical outcome, is reduced by enoxaparin in comparison to unfractionated heparin, which documents a better control of the platelet–endothelium interaction with enoxaparin, an effect less apparent with dalteparin in similar conditions.

The vWF and thrombin inhibition in unstable angina. The vWF mediates platelet adhesion to sites of vascular damage through the glycoprotein-Ib receptor, acts as a platelet agonist, and binds to glycoprotein-IIb/IIIa receptor, facilitating platelet aggregation (22,23). Moreover, vWF is bound to factor VIII, protecting it from inactivation and delivering it at the sites of the damaged vessel (24). More vWF provides more factor VIIIa available for both Xa and thrombin generation. Thrombin plays a pivotal role in the thrombotic process through its numerous activating effects on coagulation and platelets. Thrombin is a major agonist of both endothelial cells and platelets, then causing more vWF release from these activated cells. Our data obtained with PEG-hirudin, a direct thrombin inhibitor with long-lasting effects, confirm further the hypothesis that a good control of thrombin is associated with little vWF release, an indicator of favorable outcome at one-month follow-up.

Our data are also consistent with the platelet effects measured ex vivo with another direct thrombin inhibitor (argatroban) (21) and with the clinical results of recent trials opposing hirudin to unfractionated heparin in unstable angina. In OASIS-2, lepirudin was superior to unfractionated heparin in preventing death and myocardial infarction at day 3, which was confirmed at days 7 and 35 with a combined analysis of OASIS-1 and -2 trials (4,5). The preliminary short-term results obtained with PEG-hirudin opposed to unfractionated heparin in a phase-2 trial showed similar trends with a 62% reduction of death and myocardial infarction (NS) and a 75% reduction of the triple end point, including revascularizations (p = 0.03) (7).

Anticoagulants and vWF release in unstable angina. The platelet effects of heparins have gained little attention in the management of unstable angina, and the proaggregant effects of unfractionated heparin, despite the use of aspirin, may well be clinically relevant, contributing to refractory ischemia or rebound effect after heparin discontinuation (25). Release of vWF seems to be a good marker of the control exerted by anticoagulant treatments on platelet activation as well as a marker of prognosis. Such a marker may help identify high-risk patients and control the efficacy obtained with the new standards of anticoagulation in unstable angina. The main limitation of our study is the lack of randomization among the four treatment groups; however, our patients were enrolled in various randomized trials testing the different anticoagulants, and biological analyses
were blinded for clinical outcome and treatment allocation. Our ongoing French multicenter ARMADA trial randomizes unstable angina patients to receive either unfractionated heparin, enoxaparin, or dalteparin and will examine the vWF hypothesis as a secondary end point of the study.

Conclusions. In summary, the early increase of vWF in unstable angina patients treated with unfractionated heparin is blunted by PEG-hirudin or enoxaparin but little reduced by dalteparin. Considering the pathogenetic role and prognostic value of vWF, this platelet effect may be an important explanation for the superiority observed with enoxaparin and direct thrombin inhibitors in clinical trials.

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