Myocardial damage, inflammation and thrombin inhibition in unstable coronary artery disease

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Revised 20 April 2002; accepted 24 April 2002

Aim Unstable coronary artery disease (CAD) is a multifactorial disease involving both thrombotic and inflammatory processes. We have assessed the time-course and the influence of thrombin inhibitors on changes in fibrinogen and C-reactive protein levels, and their relation to myocardial ischaemia in unstable CAD.

Methods and results Three hundred and twenty patients were randomized to 72 h infusion with three different doses of inogatran, a direct thrombin inhibitor, or unfractionated heparin. There were no significant differences between the treatment groups in fibrinogen or C-reactive protein levels. Overall, the fibrinogen levels were significantly increased in the first 24–96 h and still elevated at 30 days. The C-reactive protein levels showed a more pronounced increase during the first 24–96 h, but then markedly decreased over 30 days. Troponin-positive compared to troponin-negative patients had higher fibrinogen and C-reactive protein levels up to 96 h, although there was an increase compared to pre-treatment levels in both groups. A high fibrinogen level (pre-treatment top tertile) was associated with an increased rate of death or myocardial (re-)infarction at 30 days, 13% vs 5.6%, P = 0.03, and increased long-term mortality. A high C-reactive protein level was related to increased 30-day mortality, 4% vs 0%, P = 0.01.

Conclusion Myocardial cell injury was related to a high degree of inflammation, only some of which is an acutephase response due to tissue damage. The rise in fibrinogen was sustained, which might reflect low grade inflammation with long-term risk of thrombosis. The transient elevation of C-reactive protein levels might indicate a propensity to a pronounced inflammatory response and is associated with increased mortality.

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KEYWORDS Unstable coronary artery disease; inflammation; fibrinogen; C-reactive protein; troponin

Introduction Unstable coronary artery disease, i.e. unstable angina or non-Q-wave myocardial infarction, is a multi-factorial disease. Exposure of the thrombogenic contents of a ruptured or fissured atherosclerotic plaque triggers platelet and coagulation activation which may subsequently lead to thrombus formation. Furthermore, by destabilizing the atherosclerotic plaque and enhancing thrombus formation, inflammatory processes may also be

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0195-668X/02/$ - see front matter © 2002 The European Society of Cardiology. Published by Elsevier Science Ltd. All rights reserved. doi:10.1016/S0195-668X(02)00312-3
involved in the initiation of unstable coronary artery disease. At autopsy, active inflammation is evident by the accumulation of macrophages at sites of the plaque rupture. Moreover, elevated levels of interleukins, acute-phase proteins, activated circulating monocytes and lymphocytes have been reported from clinical studies in unstable coronary artery disease.

Fibrinogen is an acute-phase protein directly involved both in platelet aggregation by cross-linking the glycoprotein Ib/IIa-receptors on adjacent platelets, and in the coagulation cascade. C-reactive protein, another acute-phase protein, has an unclear biological function, but it has been suggested that C-reactive protein may also interact in the atherosclerotic process by activation of the complement system. The early levels of fibrinogen and C-reactive protein have in several studies been identified as indicators of increased short- and long-term risk for adverse clinical outcome in unstable coronary artery disease. There is limited knowledge about the time-course and the influence of anticoagulant treatment of changes in fibrinogen and C-reactive protein in unstable coronary artery disease.

The Thrombin Inhibition in Myocardial Ischemia (TRIM) study enrolled unstable coronary artery disease patients in Scandinavian centres during 1994 and 1995. The patients were randomized to three different doses of inogatran, a low molecular mass direct thrombin inhibitor, or standard unfractionated heparin, given as intravenous infusion for 72 h. The aim of the present substudy was to assess the degree of inflammatory activity, as reflected by the course of changes in fibrinogen and C-reactive protein levels in serial plasma samples, the influence of unfractionated heparin or different doses of inogatran on these levels, and their relation to manifestations of myocardial ischaemia.

Methods

Patients and design

The substudy population consisted of 320 consecutive patients recruited in 19 of the 61 participating centres of the TRIM study. Details of the TRIM study protocol and the main results have previously been reported. Eligible for inclusion were men and post-menopausal women between 25 and 80 years of age with unstable angina, defined as new onset of ischaemic chest pain or rapid deterioration in previously stable angina during the last 4 weeks, or suspicion of a non-Q-wave myocardial infarction. This clinical diagnosis had to be supported by either changes in the resting ECG, e.g. ST-depression or T-wave inversion, or previously known coronary artery disease.

Patients were, within 24 h from the qualifying episode of chest pain, randomized to blinded treatment with unfractionated heparin or one of three different fixed doses of inogatran. Low, medium and high dose inogatran patients received intravenous bolus injections of 1.10; 2.75 and 5.50 mg respectively, followed by continuous infusion of 2.0; 5.0 and 10.0 mg . h⁻¹ respectively. Heparin was administered as a 5000 U intravenous bolus injection followed by infusion with 1200 U . h⁻¹. All infusions were to be continued for 72 h. Aspirin was strongly recommended and given to 96% of the patients within the first day, but other platelet inhibitors and oral anticoagulants were not allowed.

Fibrinogen, C-reactive protein and troponin T

Venous blood samples were obtained, preferably by direct venipuncture, into citrated tubes for analyses of fibrinogen and C-reactive protein and into heparin tubes for analysis of troponin T. Samples for analyses of fibrinogen and C-reactive protein were collected pre-treatment, during study drug infusion at 24 and 72 h and 24 h after cessation of infusion at 96 h, and finally at 30 days follow-up. For the analysis of troponin T, samples were drawn pre-treatment, at 6 and 12 h. The first 2 ml blood were disposed of and the samples were within 30 min centrifuged at 2000 g for 20 min. Aliquots of 500 µl plasma in Eppendorf tubes were frozen and stored at –70°C until analysis.

Fibrinogen was analysed by rate nephelometry with a Beckman Array protein system (Beckman Instruments Inc). The assay was performed according to the recommendations of the manufacturer, except that goat antihuman fibrinogen (Atlantic Antibodies) was used. The assay was calibrated against a human plasma standard (Behring Diagnostics GmbH). C-reactive protein was analysed with the Immulite system, a chemiluminescent enzyme-labelled immunometric assay based on a ligand-labelled monoclonal antibody and separation by antiligand-coated solid phase (Immulite CRP, Diagnostic Products Corporation). Measurements of troponin T (ELISA Troponin(e) T) were carried out with an ES 300 analyser (Boehringer Mannheim GmbH). The discriminator value for myocardial cell injury recommended by the manufacturer was 0.1 µg . l⁻¹, but
evaluations were also performed using 0.06 µg \cdot l^{-1} as cut-off.

End-points

Clinical end-points were a composite of death or nonfatal myocardial (re-)infarction at 72 h (the end of infusion), 7 and 30 days. Myocardial (re-)infarction was diagnosed using standard clinical, ECG and cardiac marker criteria. An independent end-point committee evaluated all end-points.

Long-term follow-up data were obtained from 286 of the 320 patients at a median of 29 months (range 12–50 months). This information was obtained from hospital records and local or national registries. If data in these sources were missing the information was collected by telephone interview.

Statistics

Differences in the levels of inflammation markers were judged with non-parametric tests, between-group comparisons with Mann-Whitney or Kruskal-Wallis tests as appropriate, and within-group differences between different time-points with Wilcoxon signed rank tests. The levels of inflammatory markers and their relation to clinical outcome were evaluated for the total substudy population. Fisher exact test (two-sided) or chi$^2$ tests as appropriate were used to judge significance of differences in proportions.

Results

There were no significant differences between patients in the four treatment groups in levels of fibrinogen or C-reactive protein either pre-treatment, during the 72 h of anticoagulant treatment, or at 24 h and 30 days thereafter. There were furthermore no significant differences between heparin and the combination of the three inogatran groups, or between any of the three inogatran groups concerning the composite end-point after 7 or 30 days.

Baseline characteristics

Taking all treatment groups together, patients with high pre-treatment fibrinogen levels, i.e. in the top tertile, were older (median 68 vs 65 years, $P = 0.003$) and had in higher proportion a history of stable angina >4 weeks (74% vs 62%, $P = 0.04$), previous myocardial infarction (55% vs 42%, $P = 0.03$) and congestive heart failure (26% vs 8%, $P<0.001$). Hypertension and diabetes mellitus were present in 39% and 17% of the patients, respectively, without differences in relation to pre-treatment fibrinogen levels. There were no differences in baseline characteristics in relation to pre-treatment C-reactive protein levels.

Time-course of changes in fibrinogen and C-reactive protein levels

Median time from onset of chest pain to randomization was 12 h, and 20% of the 320 patients were randomized within 6 h of symptom onset. Overall there was a significant increase in fibrinogen during the first 24 h after randomization, which seemed to reach its maximum after 72–96 h (Table 1). After 30 days the fibrinogen levels still remained significantly higher than pre-treatment. The increase in C-reactive protein levels was, as compared to changes in fibrinogen levels, more pronounced with a large upward dispersion during the first 24–96 h (Table 1). In contrast to fibrinogen, the levels of C-reactive were markedly decreased from day 4 to day 30, when the level tended to be lower than pre-treatment.

Fibrinogen and C-reactive protein in relation to troponin T

Pre-treatment elevation of troponin T≥0.1 µg \cdot l^{-1} was found in samples from 138 (44%) of the 317

<table>
<thead>
<tr>
<th>Table 1 Time-course of changes in fibrinogen and C-reactive protein</th>
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<tbody>
<tr>
<td><strong>Fibrinogen g.l^{-1}, n = 314</strong></td>
</tr>
<tr>
<td>Pre-treatment</td>
</tr>
<tr>
<td>24 h</td>
</tr>
<tr>
<td>72 h</td>
</tr>
<tr>
<td>96 h</td>
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<tr>
<td>30 days</td>
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<tr>
<td><strong>C-reactive protein mg.l^{-1}, n = 306</strong></td>
</tr>
<tr>
<td>Pre-treatment</td>
</tr>
<tr>
<td>24 h</td>
</tr>
<tr>
<td>72 h</td>
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<tr>
<td>96 h</td>
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<tr>
<td>30 days</td>
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</table>

Values are medians (first and third quartiles).

*P<0.001 and †P = 0.08 for the difference compared to pre-treatment levels. Wilcoxon signed rank test.
analysed patients. These troponin-positive patients had significantly higher levels of fibrinogen and C-reactive protein pre-treatment and at 24–96 h (Fig. 1). In both troponin-positive and troponin-negative patients there was a significant further increase in levels of fibrinogen and C-reactive

Fig. 1 Distribution of (a) fibrinogen or (b) C-reactive protein in relation to pre-treatment levels of troponin T<0.1 µg l⁻¹ (white boxes) or ≥0.1 µg l⁻¹ (shaded boxes). Box-plots contain median, first and third quartiles and in the whiskers 10th and 90th percentiles. Mann-Whitney test for between-group comparisons, Wilcoxon signed rank test (within-group) compared to pre-treatment, *P<0.05, **P<0.01, ***P<0.001.
protein during the first 24–96 h (Fig. 1). This increase in levels of fibrinogen and C-reactive protein in troponin-negative patients was still significant when excluding patients with clinical events, i.e. death or myocardial (re-)infarction during the study.

Similar results were found using 0.06 µg l⁻¹ as cut-off limit for pre-treatment troponin T or positive troponin T in serial samples within the first 12 h.

**Clinical outcome in relation to fibrinogen and C-reactive protein**

High fibrinogen levels, i.e. in the pre-treatment top tertile, were related to increased risk of ischaemic events at 30 days (Table 2). However, the rate of adverse ischaemic events tended to be lower during the ongoing anticoagulant treatment in patients with high pre-treatment fibrinogen, but within the first 4 days after cessation of treatment there was a clinical reactivation with a more than doubled ischaemic event rate (Fig. 2). Thus, 12 of the 100 patients with pre-treatment levels of fibrinogen in the top tertile (and without ischaemic events during treatment) died or experienced a myocardial (re-)infarction from cessation of treatment to 30-days follow-up, as compared to seven (3.4%) of the 208 patients with lower pre-treatment fibrinogen levels, \( P = 0.003 \). Furthermore, a high pre-treatment level of fibrinogen was a predictor of long-term mortality (Fig. 3).

Pre-treatment C-reactive protein levels were not related to the composite of death or myocardial (re-)infarction during or after anticoagulant treatment. However, high pre-treatment C-reactive protein, i.e. in the top tertile, was significantly related to increased mortality at 30 days (Table 2) and associated with a trend for increased long-term mortality (Fig. 3).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical outcome at 30 days in relation to pre-treatment levels of fibrinogen and C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (g.l⁻¹)</td>
<td>C-reactive protein (mg.l⁻¹)</td>
</tr>
<tr>
<td>&lt;3.12</td>
<td>3.12–3.80</td>
</tr>
<tr>
<td>n = 105</td>
<td>n = 108</td>
</tr>
<tr>
<td>Death or MI</td>
<td>7 (6.7)</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
</tr>
</tbody>
</table>
| MI = myocardial (re-)infarction. Values shown are number of patients with percentages of the group in parenthesis. \( P \)-values are calculated by chi-square or †Fisher exact test (two-sided) for top vs bottom+middle tertile.

**Fig. 2** Composite of death and myocardial (re-)infarction in relation to pre-treatment levels of fibrinogen or C-reactive protein. Pre-treatment top tertile indicated by a solid line and bottom + middle tertile by a broken line. Vertical line indicates cessation of study drug.
Discussion

Active inflammation, reflected by elevated levels of interleukins and acute-phase proteins, e.g., fibrinogen and C-reactive protein, in unstable coronary artery disease has previously been reported. In the present study, the changes in the levels of fibrinogen and C-reactive protein had different time-courses. The fibrinogen level started to rise at 24 h, seemed to reach its maximum after 72–96 h and remained at 30 days higher than before treatment. The initial increase in levels of C-reactive protein occurred somewhat earlier and was more pronounced. However, the C-reactive protein level at 30 days tended to be lower than before treatment. Neither the time-course of levels of fibrinogen nor C-reactive protein levels seemed affected by the different doses of inogatran or UF heparin treatment. Therefore, the short-term elevation of C-reactive protein seems mainly related to a transient increase in inflammatory activity, with spontaneous resolution, while the continuous elevation of fibrinogen indicates the co-existence of a chronic low-grade inflammatory condition.

There are limited data concerning the inflammatory activity in patients with unstable angina in relation to signs of myocardial cell damage. Consistent with previous studies, the troponin-positive patients had significantly higher levels of fibrinogen and C-reactive protein pre-treatment and up to at least 96 h thereafter in the present study. This enhanced acute-phase reaction is probably induced by the myocardial cell damage.

In a previous study of unstable angina patients without troponin elevation, there was no increase in the levels of C-reactive protein up to 96 h after admission, despite ischaemic episodes during 24 h continuous ECG-monitoring in the majority of the patients. In contrast, elevated levels of C-reactive protein despite normal troponin T on admission were reported in another study of unstable angina patients. In eight of these 20 patients the C-reactive protein level was doubled at 24–72 h after admission, and all of them had an in-hospital major coronary event (death or myocardial infarction) or underwent urgent revascularization. The present study is thus the first to report a significant acute-phase response, with an early increase in fibrinogen and especially C-reactive protein, in a large number of unstable angina patients without any signs of myocardial cell damage. This acute-phase reaction indicates other sources of inflammatory activity than myocardial cell injury in the acute phase of unstable coronary artery disease. Recently, polymorphism in exon 2 of the C-reactive protein gene has been described, although no association with C-reactive protein regulation or concentration is known. One might speculate that differences in the acute-phase response in unstable coronary artery disease might reflect differences in the individual response to inflammatory stimuli. Interestingly, higher C-reactive protein levels have been reported in the offspring of patients with myocardial infarction. Thus, a low-grade inflammatory activity, indicated by slight elevation of C-reactive protein and long-lasting fibrinogen elevation, might be associated with a propensity to
a pronounced inflammatory response on plaque ruptures and/or thromboembolic myocardial damage.

Several epidemiological studies have identified elevated levels of markers of inflammation, mainly C-reactive protein, as risk indicators for future cardiovascular events, both in apparently healthy men\textsuperscript{32--35} and women.\textsuperscript{36} Furthermore, both fibrinogen and C-reactive protein have been found to indicate increased short- and long-term risk for adverse clinical outcome in unstable coronary artery disease.\textsuperscript{7,13--17}

The differences in magnitude and time-course of elevations of fibrinogen and C-reactive protein levels observed in the present study might indicate different underlying mechanisms of their associations to new ischaemic events in unstable coronary artery disease. A high fibrinogen level was related with a trend towards a lower rate of clinical events during ongoing anticoagulant treatment, but also marked clinical reactivation after cessation of treatment with a significantly higher rate of ischaemic events at 30 days. Fibrinogen is directly involved in the thrombotic process, both in platelet aggregation cross-linking the glycoprotein llb/llla-receptors on adjacent platelets, and in the coagulation cascade where it is cleaved by thrombin to soluble fibrin which subsequently polymerises to form the fibrin network stabilising the platelet clot.\textsuperscript{11} The sustained high levels of fibrinogen might thereby be associated with long-term risk of thrombosis and myocardial (re-)infarction.\textsuperscript{13}

On the other hand, the C-reactive protein level was only related to increased mortality and not to myocardial (re-)infarction in the present as in other trials.\textsuperscript{13,16,37,38} Similar to these results, elevated levels of interleukin-6 have been related to increased mortality, but not an increase in the combined end-point of death and myocardial infarction, in patients with unstable coronary artery disease.\textsuperscript{39} C-reactive protein is mainly regulated by interleukin-6, which is present in the atherosclerotic plaque and secreted by both endothelial cells, smooth muscle cells, macrophages and T-cells.\textsuperscript{40} High levels of interleukin-6 may thereby reflect a greater atherosclerotic burden and/or increased inflammatory activity in the plaques. Thus, these plaques would be more vulnerable and prone to deeper fissuring, causing more severe thrombotic episodes.\textsuperscript{39} However, the largest increase in C-reactive protein was observed in patients with myocardial cell injury. Also the raised mortality was mainly seen in the top tertile of C-reactive protein. Therefore, much of the relation between the C-reactive protein level and mortality could be explained by an inflammatory reaction in the damaged myocardium.

In conclusion, myocardial cell injury, as detected by elevated troponins, was associated with a higher degree of inflammation, only in part explained by an acute-phase response due to the tissue damage. The elevation of fibrinogen levels in the acute phase was sustained for at least 30 days, which might reflect a low grade inflammatory condition in atherosclerotic lesions and a pro-thrombotic state, thus explaining the association between the fibrinogen level and the long-term risk for thrombosis and new ischaemic events. This risk seems diminished during treatment with thrombin inhibitors, but recurs early after cessation of treatment. The pronounced elevation of C-reactive protein levels in the acute phase was transient, which might indicate a propensity to a pronounced inflammatory response at plaque ruptures and/or thromboembolic myocardial damage, both of which might be associated with increased mortality. The mechanisms of the inflammatory response in the acute phase of unstable coronary disease need to be further explored in order to understand their association with future cardiovascular incidents.

Acknowledgements

This study was supported by AstraZeneca AB, Mölndal, Sweden and by grants from the Swedish Heart and Lung Foundation, Uppsala County Association against Heart and Lung Diseases, and the Faculty of Medicine, Uppsala University. We acknowledge Birgitta Fahlström for excellent technical assistance.

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