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Inhibitors of the Hemostasis and Related Systems in Patients with Acute Myocardial Infarction or Unstable Angina Pectoris

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SUMMARY

***Background*: alterations of the hemostatic and fibrinolytic systems are known in patients with acute coronary syndromes.**

***Objective:* to investigate the meaning of several inhibitors of these systems in patients with acute myocardial infarction (n = 20) and unstable angina pectoris (n = 21).**

Methods: repeated venous blood sampling was performed during the first 10 days after admission. Plasma inhibitors were measured using chromogenic substrate tests. Data were compared with controls (n = 25).

***Results:* the C_1-esterase inhibitor did not show a significant difference to the controls, but in patients with unstable angina pectoris a slight intraindividual increase during the follow-up was observed. α_2-macroglobulin in contrast was transiently reduced in comparison with the controls ($p < 0.05$) in patients with unstable angina pectoris, whereas no significant alterations were found in patients with acute myocardial infarction. α_1-antitrypsin increased in both groups significantly during the observation period indicating an acute phase reaction. Antithrombin III was slightly decreased in patients with acute coronary syndromes during the 10 day follow-up. No significant alterations were found in α_2-antiplasmin and protein C levels.**

***Conclusion*: our results indicate that besides the known alterations of the plasminogen activator inhibitor in the fibrinolytic system the other inhibitor systems are only slightly involved. The borderline alterations indicate consumption of some of the inhibitors due to the known activation of thrombin generation or the kallikrein-kinin system, but do not indicate that an inhibitor deficiency is a reason for the activation of the coagulation in acute coronary syndromes.**

Disturbances of the hemostatic and fibrinolytic systems are well known findings in patients with acute coronary syndromes and intracoronary thrombus formation is frequently observed in these patients.[1,2] In patients with acute coronary syndromes alterations of the plasminogen activator inhibitor system were described.[3–5] Recently it was demonstrated that, besides the disturbed fibrinolytic system, the hemostasis is activated in these patients resulting in a hypercoagulative state.[6] Furthermore, the kallikrein-kinin-contact phase system was found to be activated in patients with unstable angina pectoris.[7] In order to investigate whether, besides the known alterations of the plasminogen activator inhibitor, additional inhibitors of the hemostasis and related systems are involved in the pathogenesis of acute coronary syndromes, we examined plasma of patients with acute myocardial infarction or unstable angina pectoris during the first 10 days after admission to the hospital.

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PATIENTS AND METHODS

41 patients with acute coronary syndrome were included into the study. 20 patients had an acute myocardial infarction and 21 had unstable angina pectoris. All patients

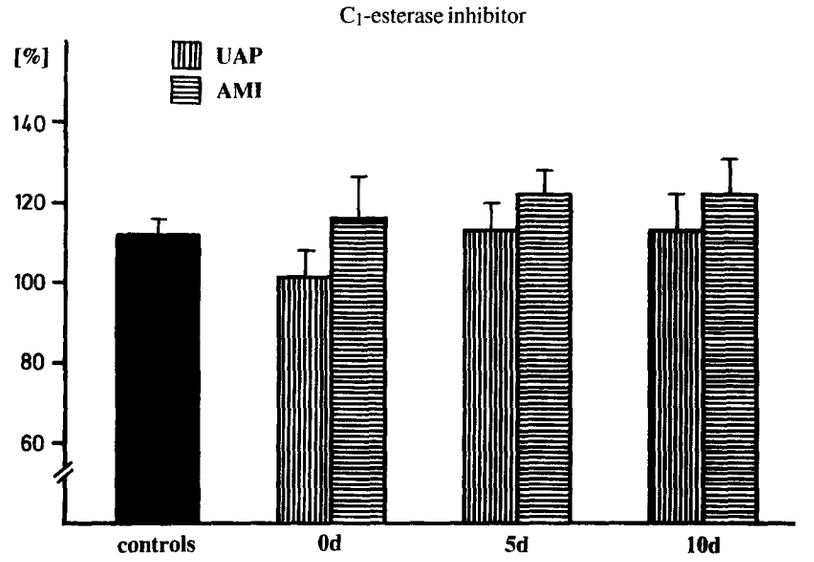

Fig. 1 C_1-esterase inhibitor in patients with unstable angina pectoris (UAP) and acute myocardial infarction (AMI). Means ± SEM.

were referred to the intensive care unit of our institution for treatment for acute myocardial infarction or severe unstable angina pectoris. The age of the patients ranged from 31–83 years (mean age acute myocardial infarction group: 64 ± 2 years; unstable angina pectoris group: 57 ± 3 years). 32 men and 9 women were included into the study. The majority (74%) had a coronary angiography, in the remaining patients typical ECG signs of an acute or prior transmural myocardial infarction were present. In patients with acute myocardial infarction, diagnosis was confirmed by the creatine kinase time course and repeated ECG registrations. In contrast, patients with unstable angina pectoris did not have significantly elevated creatine kinase levels during the repeated measurements and did not develop signs of a Q-wave myocardial infarction. Patients with unstable angina pectoris were classified into class IIIb and class IIIc of the Braunwald classification.[8] Treatment of all patients was initiated with intravenous heparin therapy (1000 IU/h) controlled by repeated measurements of the aPTT. Intravenous heparinization was continued for 24–48 h and was stopped one day after stabilization of the symptoms. Thereafter, the patients received 7500 IU heparin b.i.d. subcutaneously. The intravenous heparinization was adjusted to obtain a prolongation of the aPTT to 2-times the upper range of normal. The heparin levels found in the plasma using this management were below 0.5 U/ml. Such low heparin levels do not critically disturb the analytical methods applied in the present study.

Furthermore, the patients received nitrates, calcium

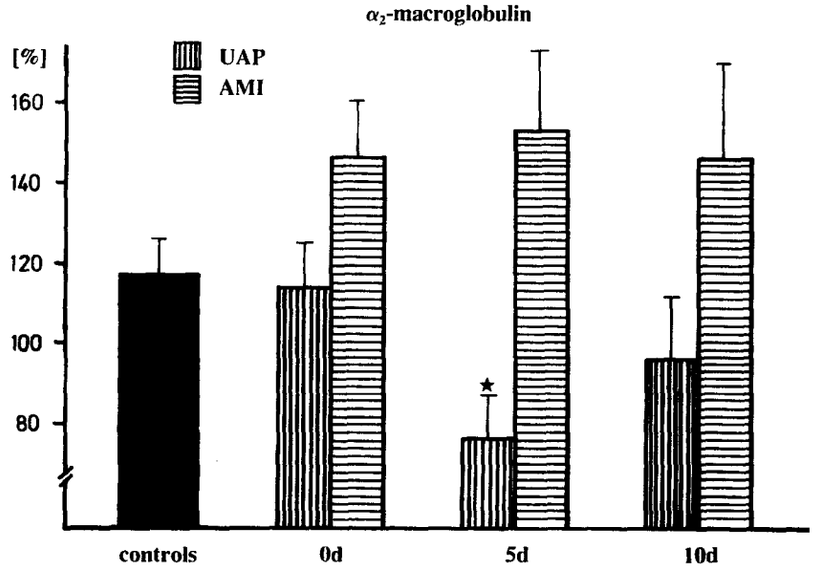

Fig. 2 α_2-macroglobulin in patients with unstable angina pectoris (UAP; transient reduction) and acute myocardial infarction (AMI). Means ± SEM; *= $p < 0.05$ vs controls.

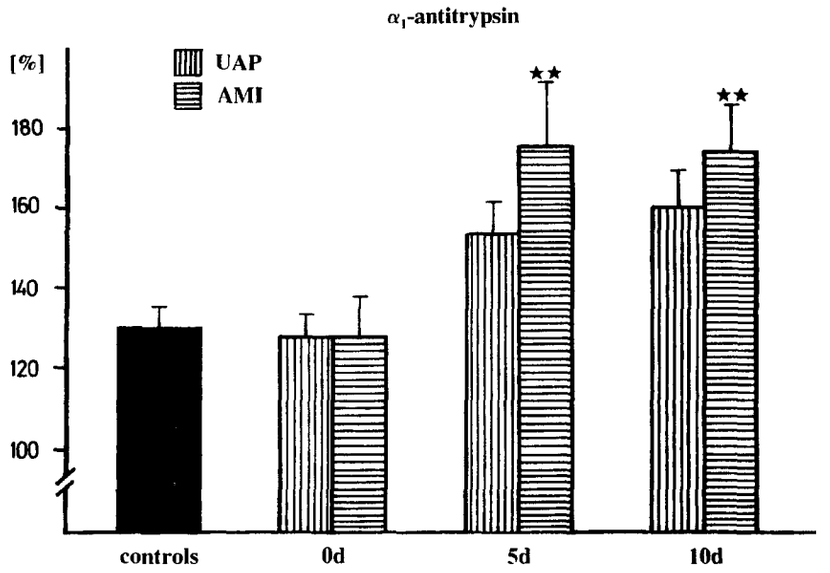

Fig. 3 α_1-antitrypsin demonstrating an acute phase reaction pattern in patients with unstable angina pectoris (UAP) and acute myocardial infarction (AMI). Means ± SEM; ** = $p < 0.01$).

antagonists and betablockers, as well as aspirin, depending on the individual need for treatment. Patients with unstable angina pectoris, who were not stabilized within the first hours of treatment on the intensive care unit and who needed revascularization by PTCA or CABG, were not included into the follow-up study. Patients with acute myocardial infarction, who received a thrombolytic therapy, were also not included. Since angiography might influence some of the systems investigated,[9] no blood was obtained within a time interval of at least 2 h after coronary angiography.

Blood sampling was performed on admission at the intensive care unit, 3 h after admission, 9 h after admission, 24 h after admission and 2, 5 and 10 days after the first blood sampling. Except for the first samples, which were obtained at admission, the further blood sampling was performed between 7 and 8 a.m. to exclude a possible diurnal variation. The venous blood was sampled in 10 ml citrated vials, afterwards the blood was centrifuged at 2000 *g* and 20°C for 20 min. Aliquots were afterwards shock-frozen in liquid nitrogen.

As reference group, 25 patients with a mean age of 48 ± 4 years (range 21–83 years) were examined. None of these persons had evidence of a cardiovascular disease and all of them were free of cardiac events for a 2-year follow-up period. All control persons were non-smokers (12 women and 13 men).

C_1-esterase inhibitor was measured using a commercially available chromogenic substrate test (C_1-1: Immuno, Vienna, Austria). Determination of α_1-antitrypsin was done using a chromogenic substrate test with the sub -strate A-2677 from Kabi Vitrum (Moelndal, Sweden).

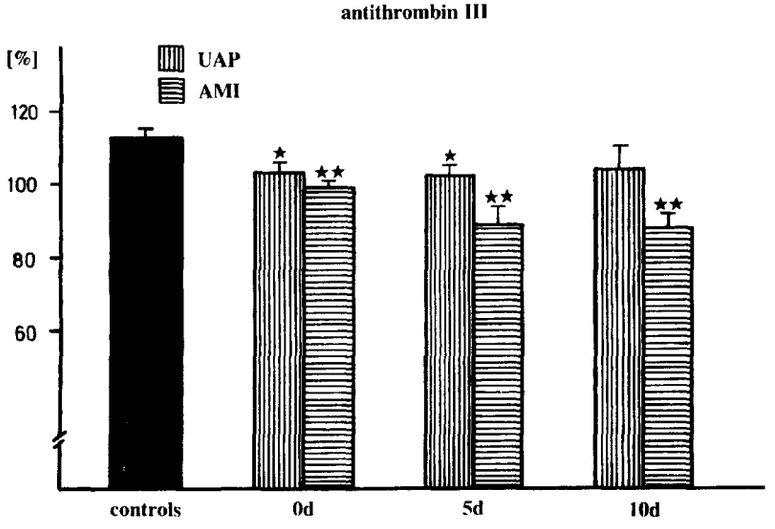

Fig. 4 Antithrombin III in patients with unstable angina pectoris (UAP) and acute myocardial infarction (AMI) with a persistent moderate reduction in both groups (means ± SEM: * = $p < 0.05$, ** = $p < 0.01$).

α_2-macroglobulin was measured using a chromogenic substrate (S-2677) test from Kabi Vitrum (Moelndal, Sweden). For determination of α_2-antiplasmin, a commercially available kit (Kabi Vitrum GmbH, Munich, Germany) with chromogenic substrate (S-2251) was used. Antithrombin III was determined using a chromogenic substrate (S-2238) test from Kabi Vitrum (Moelndal, Sweden). Measurement of protein C was done using a commercially available chromogenic substrate (S-2366) test (Kabi Vitrum, Moelndal, Sweden). All measurements were performed in duplicate and the mean values were reported.

Statistical evaluation was done using the SPSS software package (SPSS Inc., Chicago, IL, USA). Data from patients were compared with the controls using the unpaired two-tailed version of Student's t-test. To test for a possible increase or decrease of a parameter during follow-up mean values of the samples from the first day were compared to means of the samples from the second to tenth day of the follow-up using a paired version of Student's t-test. The level of significance was set at $p < 0.05$.

RESULTS

In patients, both with unstable angina pectoris and with acute myocardial infarction, alterations of the C_1-esterase inhibitor levels compared with the controls were not observed (Fig. 1). Nevertheless, there was a significant ($p < 0.05$) increase of the C_1-esterase inhibitor levels within the group with unstable angina pectoris during follow-up.

In patients with unstable angina pectoris, a transient reduction in α_2-macroglobulin levels during the follow-up with significantly ($p < 0.05$) lower values until 5 days after admission was found in comparison with the control group (Fig. 2). In patients with acute myocardial infarction, α_2-macroglobulin tended slightly to a higher level without any change during the follow-up.

The levels of α_2-antiplasmin in patients with unstable angina pectoris and with acute myocardial infarction were identical to the data of control persons ($111 \pm 5\%$ vs $114 \pm 5\%$ at admission; $116 \pm 7\%$ vs $123 \pm 6\%$ at day 5; $111 \pm 7\%$ vs $121 \pm 6\%$ at day 10; controls $115 \pm 5\%$).

Initial measurements of α_1-antitrypsin in patients with acute coronary syndrome were comparable to controls. During the follow-up, significantly higher α_1-antitrypsin levels were observed in patients with acute myocardial infarction ($175 \pm 12\%$; Fig. 3), whereas in patients with unstable angina pectoris, the increase was less marked ($161 \pm 9\%$) but also detectable in the intraindividual comparison ($p < 0.05$).

Antithrombin III was initially slightly decreased compared with controls in patients with unstable angina pectoris (Fig. 4). In patients with acute myocardial infarction after a significant initial reduction in antithrombin III levels a further moderate decrease was observed.

Determination of protein C did not show any significant alterations in patients with acute coronary syndromes (neither in comparison with controls nor intraindividually) ($130 \pm 5\%$ vs $120 \pm 7\%$ at admission; $132 \pm 7\%$ vs $132 \pm 4\%$ at day 5; $131 \pm 13\%$ vs $138 \pm 5\%$ at day 10; controls $124 \pm 6\%$).

DISCUSSION

Intracoronary thrombus formation at the site of the severely diseased coronary endothelium resulting in a critical stenosis or complete occlusion of a coronary vessel is frequently observed in acute coronary syndromes.[1,2] Alterations of the plasminogen activator inhibitor system were described in these patients.[3–6]

In prior investigations, an activation of the kallikrein-kinin system in patients with unstable angina pectoris was found.[7] We therefore determined the C_1-esterase inhibitor, which is the main inhibitor of the kallikrein system,[10] and the main plasma inhibitor of tissue-type-plasminogen activator[11] and of factor XIIa.[10] Its initial borderline reduction in patients with unstable angina pectoris is indicative of a consumption of this inhibitor due to the above mentioned activation of the kallikrein-kinin system and the contact phase or due to elevated tissue-type-plasminogen activation levels in such patients.[6] During the 10 day follow-up, C_1-esterase levels increased. Other authors observed slightly elevated C_1-esterase levels early[4] and even months after an acute myocardial infarction in female patients.[12]

A further, but less important inhibitor of the kallikrein system, is α_2-macroglobulin.[13] Gram and Jespersen[14] did find normal levels of α_2-macroglobulin in patients with acute myocardial infarction and additional venous thrombosis as well as in patients with acute myocardial infarction without venous thrombosis. While Almèr and Öhlin[15] reported a borderline elevation of this inhibitor, we observed a minor reduction in α_2-macroglobulin in patients with unstable angina pectoris, which was not observed in patients with acute myocardial infarction in the present study. It might be speculated whether repeated periods of ischemia at rest might induce a higher degree of consumption of this inhibitor compared with a myocardial infarction with occluded coronary artery.

A typical acute phase reaction was found for α_1-antitrypsin. A delayed increase of this inhibitor, which acts for example on the thrombin system but only little on the kallikrein system,[13] was also observed by other authors in patients with acute coronary syndrome.[16,17]

In contrast to the observation of normal α_2-antiplasmin levels,[14,15] Gidron et al[17] found elevated α_2-antiplasmin levels 4–5 days and Hamsten et al[12] even later after acute myocardial infarction. In the present study, there was only a trend to elevated levels during the 10 day follow-up in patients with unstable angina pectoris and no alterations at all were found in patients with acute myocardial infarction. The difference to some of the prior studies might be explained by the relatively small number of patients included in our analysis. Furthermore, the borderline changes indicate the minor func-

tional importance of the alterations described in some of the prior studies.

Antithrombin III levels were persistently decreased in patients with acute coronary syndrome. During the initial 1–2 days, an interference with intravenous heparin therapy might be discussed, but such a possible interference would not explain the prolonged reduction in antithrombin III. An explanation could be the described increased thrombin generation in patients with acute myocardial infarction,[5] since a relation between formation of thrombin/antithrombin III complexes (due to enhanced thrombin generation) and reduced levels of antithrombin III in acute coronary syndromes was supposed.[18] The reduction in antithrombin III in the present study is not in the range of a marked deficiency of this inhibitor, but more an indicator of a slightly increased consumption. Furthermore, in patients with stable angina pectoris, a minor reduction in antithrombin III levels was observed in the ECAT-study.[19] Discrepancies in prior studies, which described either no alterations or a decreased antithrombin III, might be due to different methods used for determination of antithrombin III according to Losito et al.[16]

We did not observe significant changes in protein C, which interacts with plasminogen activator inhibitor, in the patients with acute coronary syndrome. Our results are in accordance with the known stable protein C levels in patients with acute myocardial infarction.[14]

While a causal role of the plasminogen activator inhibitor in the pathogenesis of acute coronary syndromes is discussed,[3,5,6] we did not measure alterations of other inhibitors to such an extent that these changes might be a cause for coronary thrombosis. The alterations found are, in part, indicative of a consumption due to the activation of systems involved (e.g. consumption of C_1-esterase inhibitor due to activation of the kallikrein system). Similarly, a reduction in antithrombin III levels is probably due to enhanced thrombin generation, but antithrombin III levels in the range observed in the present study are not below a critical level. Other inhibitor systems, like α_1-antitrypsin, react as acute phase proteins. Therefore, the activation of the coagulation as well as of related systems in patients with unstable angina pectoris and acute myocardial infarction cannot be explained by the only moderately altered levels of some inhibitors (except plasminogen activator inhibitor) during the first hours after admission to the intensive care unit. Nevertheless, the changes found are pointing at an enhanced consumption of inhibitors, indicating the involvement of some of the investigated systems in the pathogenesis of acute coronary syndromes.

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