Plasminogen Activator Inhibitor 1 in Platelets - Studies of Synthesis, Activity, and Glycosylation Patterns

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Abstract

Plasminogen activator inhibitor 1 (PAI-1) is the main physiological inhibitor of tissue-type plasminogen activator. Thus PAI-1 plays an important role in decreasing the fibrinolytic activity in human blood. PAI-1 is present in high concentrations in platelets and also in low concentrations in plasma, but the source of plasma PAI-1 is not known. Previous studies have shown that the activity of PAI-1 in platelets is low and this finding is not in accordance with its observed role in clot stabilisation. The aim of this thesis was to investigate the role of platelets in inhibition of fibrinolysis, and in particular the physiological regulation of platelet-derived PAI-1; its synthesis, activity, and potential contribution to plasma levels.

Investigations of mRNA levels and PAI-1 protein synthesis showed that platelets, despite their lack of nucleus, have an on-going synthesis of PAI-1. The amount of PAI-1 increased on average by 25% in 24 hours and the synthesis could be further stimulated by thrombin. Importantly, the synthesized PAI-1 was active for at least 24 hours as shown by a functional assay. There were large inter-individual variations of the synthesis rate and we therefore studied if the common 4G/5G promoter polymorphism was the cause of the variations. However, the polymorphism did not influence the expression as showed by analysis of platelet PAI-1 mRNA and protein levels in 38 men homozygous for either allele.

Previous studies reporting low platelet PAI-1 activities have been performed using different preanalytical preparatory procedures potentially causing an inactivation of PAI-1 before the activity analysis. We reinvestigated the activity of platelet PAI-1 by lysis of platelets in the presence of tPA and subsequent detection of tPA-PAI-1 complex. Our results show that the choice of lysis method and preparatory procedures is critical for the result and the activity was found to be approximately 70%. This result is in better agreement with the observed role of platelet PAI-1 in clot stabilisation.

The amount of PAI-1 synthesized in 24 hours in our *in vitro* experiments suggests that a release of as little as 3% of newly synthesized PAI-1 from platelets would be sufficient to maintain normal plasma levels. We therefore wanted to elucidate if the platelets could be the source of plasma PAI-1. Investigations of the glycosylation patterns of PAI-1 synthesized by different tissues were performed to elucidate if differences in this pattern could reveal the source of plasma PAI-1. The results suggest that platelets are the source since no glycans were found on PAI-1 from neither plasma nor platelets. Conversely, PAI-1 from the other tissues studied expressed heterogeneous glycosylation patterns. Interestingly, we also found that the raised plasma PAI-1 levels found in obese subjects is due to a contribution of PAI-1 from the adipose tissue. Obese subjects had highly glycosylated plasma PAI-1 and several of the identified glycans were found on PAI-1 from adipose tissue.

In conclusion, these findings may clarify the previous irreconcilable findings of the role of platelet PAI-1 in clot stabilization. The high levels of active PAI-1 and the continuous production of large amounts of active PAI-1 in platelets could be a mechanism by which platelets contribute to stabilization of blood clots. The results also suggest that platelets may contribute to the PAI-1 plasma levels.

Keywords: PAI-1, platelets, plasma, fibrinolysis, synthesis, polymorphism, glycosylation, activity, platelet mRNA.

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I Brogren H, Karlsson L, Andersson M, Wang L, Erlinge D, Jern S. Platelets synthesize large amounts of active plasminogen activator inhibitor 1.
Blood 2004;104:3943-48.

II Brogren H, Wallmark K, Jern S, Karlsson L. Plasminogen activator inhibitor 1 expression in platelets is not influenced by the 4G/5G promoter polymorphism.
Thrombosis Research 2007;Sep 18 [Epub ahead of print].

III Brogren H, Wallmark K, Deinum J, Karlsson L, Jern S. Preparatory procedures may lead to underestimation of platelet PAI-1 activity.
In manuscript.

IV Brogren H, Sihlbom C, Wallmark K, Lönn M, Deinum J, Karlsson L, Jern S. Heterogeneous glycosylation patterns of human PAI-1 may reveal its cellular origin.
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