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REDUCED APC CATALYZED INACTIVATION OF FACTOR Va BOUND TO STORAGE INDUCED MICROPARTICLES

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Background: Platelet concentrates contain microparticles (MP) and their numbers increase during storage. We established in a prothrombinase assay that factor V(a) was present on the surface of storage induced MP.

Aim: In this study we compared activated protein C (APC) catalyzed inactivation of this MP-bound FVa to inactivation of purified plasma FVa on phospholipid vesicles and to inactivation of platelet-derived FVa bound to thrombin activated platelets.

Methods: MP were harvested from outdated platelet concentrates. The inactivation of FVa by APC was performed in the presence of either storage induced MP (without added FVa), synthetic PtdSer containing vesicles (with purified plasma FVa) or thrombin-activated platelets (without added FVa). The inactivation of FVa was started by addition of APC (0.5nM). At selected time points, samples of the inactivation mixture were assayed for residual FVa activity in a prothrombinase assay.

Results: Purified plasma FVa was rapidly inactivated in the presence of synthetic vesicles, with only 5% \pm 4% residual FVa activity after 20 min. APC-catalyzed inactivation of MP-bound FVa resulted in 38% \pm 2% residual FVa activity and residual activity of FVa bound to thrombin-activated platelets was 25% \pm 3% (N=3). Addition of synthetic vesicles to MP or platelet bound FVa resulted in a residual activity of 5-10%. Furthermore, the velocity of Arg506 and Arg306 cleavage on MP by APC is reduced when compared to synthetic lipids, whereas on the platelet surface only the Arg506 cleavage site is compromised.

Conclusions: We observed that FVa bound to storage induced MP is more resistant to inactivation by APC than purified plasma FVa on a synthetic lipid surface. We hypothesize that there might be a difference in the binding of FVa on different surfaces e.g. synthetic vs platelet (derived) surfaces, rendering it more resistant to cleavage by APC. The APC resistance of factor Va bound to storage induced MP may have a beneficial clinical effect in patients who need haemostatic support (and lack sufficient platelets).