SaO021

Platelets in the Uremic Milieu - Exploring the Link between Protein Carbamylation and Platelet Dysfunction in ESRD

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INTRODUCTION: Bleeding diatheses occur frequently in patients suffering from end stage renal disease (ESRD) and can lead to serious complications particularly during invasive procedures. Despite the common understanding that the pathophysiology is multifactorial, there is evidence that uremic bleeding involves a functional defect of the main platelet glycoprotein receptor GpIIb/IIIa.

Urea, the toxic end-product of protein catabolism, is under physiological conditions in equilibrium with the reactive deamination product cyanate. Urea-derived cyanate can convert lysine residues of proteins and peptides into homocitrulline by carbamylation leading to irreversible changes of protein structure and function. Chronic urea overload greatly increases cyanate concentrations in ESRD patients.

In our study we examined the impact of carbamylation on GpIIb/IIIa mediated platelet adhesion and aggregation aiming at clarifying whether carbamylation could represent a mechanistic link between uremia and platelet dysfunction in ESRD patients.

METHODS: Membrane proteins were isolated from uremic and control platelets by differential centrifugation to quantify total homocitrulline by HPLC-MS/MS as well as to specifically detect carbamylated GpIIb/IIIa by biotin-PG labeling and Western Blot. To study platelet activation in response to different agonists after cyanate pretreatment as well as to compare activation of uremic and control platelets, Flow Cytometry was the method of choice. The impact of cyanate on platelet adhesion and aggregation was assessed in different types of microplate assays. Platelet aggregation was additionally analyzed by light transmission aggregometry.

RESULTS: Platelet exposure to cyanate inhibited activation, adhesion and aggregation while it did not alter cleavage of the main human thrombin receptor PAR-1 and CD62P or CD40L translocation. Compatibly, GpIIb/IIIa activation was significantly reduced in hemodialysis (HD) patients compared to healthy controls with no difference in PAR-1 cleavage by thrombin. Analysis of platelet membrane proteins revealed a significant level of carbamylation on both subunits of the GpIIb/IIIa complex in HD patients, while only minor GpIIb/IIIa modification was detected in healthy controls. Additionally, supplementation of free amino acids during carbamylation, which was shown to protect plasma proteins from carbamylation-induced damage in HD patients, could prevent loss of GpIIb/IIIa activity.

CONCLUSIONS: Using *in vitro* methods and clinical samples from HD patients, we demonstrated that carbamylation induces structural alterations of GpIIb/IIIa resulting in a conformational change and fibrinogen-binding defect that manifests as impaired adhesion and aggregation of uremic platelets. Additionally, biotin-PG labeling confirmed a significantly higher level of GpIIb/IIIa carbamylation in HD patients compared to healthy controls. Together, these findings clearly validate the concept of carbamylation as important factor involved in ESRD associated platelet dysfunction. The observation that the carbamylation-induced loss of GpIIb/IIIa activity could be prevented by addition of free amino acids during carbamylation suggests that administration of free amino acids during dialysis may help to normalize platelet function.