Mathematisch-Naturwissenschaftliche Fakultät
Institut für Pharmazeutische und Medizinische Chemie

HEINRICH HEINE
UNIVERSITÄT DÜSSELDORF

Heinrich-Heine-Universität Düsseldorf ✉ 40204 Düsseldorf

An alle Interessierten

Professur für Pharmazeutische und Medizinische Chemie

Univ.-Prof. Dr. Holger Gohlke

Telefon +49-211-81-13662
Telefax +49-211-81-13847
gohlke@uni-duesseldorf.de

cpclab.uni-duesseldorf.de

Düsseldorf, 30.5.2012

Heinrich-Heine-Universität Düsseldorf
Universitätsstraße 1
40225 Düsseldorf
Gebäude 26.23
Ebene 02 Raum 32

www.uni-duesseldorf.de

Einladung zum PHARMAZEUTISCHEN KOLLOQUIUM

Univ.-Prof. Dr. med. Rüdiger E. Scharf, FAHA
Dr. rer. nat. Volker R. Stoldt
Institut für Hämostaseologie, Hämotherapie und Transfusionsmedizin
Universitätsklinikum Düsseldorf

The impact of platelet integrin variants: From bedside to bench

Platelets play a pivotal role both in hemostasis and arterial thrombus formation. Specifically, circulating resting platelets respond immediately to vascular lesions by becoming adherent and by forming aggregates at sites of the injured vessel wall. Once stimulated, platelets react uniformly and do not distinguish between traumatic injury and atherosclerotic or inflammatory damage of the vessel wall. While their *physiological* function is (i) to support arrest of bleeding, (ii) to contribute to host defense and wound healing, and (iii) to restore vessel wall integrity, platelets can form occlusive thrombi as a consequence of vascular diseases, such as atherosclerosis. Thus, under *pathological* conditions, platelet responses may result in myocardial infarction, stroke, or other ischemic syndromes.

Platelet adhesion is mediated by a variety of receptors, while integrin αIIbβ3 (also known as GPIIb-IIIa) is essential for platelet aggregation and subsequent thrombus formation. Integrins are heterodimeric cell adhesion molecules that can interact both with plasma proteins and extracellular matrix components, thereby transmitting transmembrane signals from the outside to the inside of the cell and vice versa (“input-output devices”). In *resting* platelets, integrins exhibit a low affinity state for plasma ligands but shift to a high affinity state upon activation at sites of vascular injury.

Human platelet integrin αIIbβ3, the most abundant receptor in nature with about 80,000 to 100,000 copies per platelet, is *polymorphic* with a leucine or proline at residue 33 of the mature β3 subunit. The Pro→Leu exchange results from a T→C substitution at position 1565 in exon 2 of the β3 gene *(ITGB3)*. Polymorphisms are stable DNA sequence variations that occur in > 1% of chromosomes in the population. As a consequence of the Pro33→Leu33 exchange, the human platelet antigen (HPA) pattern is altered with expression of HPA-1a (Leu33) or the variant isoform HPA-1b (Pro33).

Clinical studies suggest that the HPA-1b (Pro33) variant of αIIbβ3 is a *risk factor* of acute coronary syndromes, and it has been speculated that HPA-1b (Pro33) is associated with increased platelet thrombogenicity. To test this hypothesis in further detail, we have studied > 4,000 patients with coronary artery disease and documented that those who are carriers of HPA-1b (Pro33) experience their myocardial infarction 5.2 years (median) earlier than HPA-1a/1a (Leu33) patients. Based on these findings, we have postulated that HPA-1 (Pro33) of αIIbβ3 is indeed a *prothrombotic* variant.

To examine the genotype–phenotype relation, we have studied functional and biochemical properties of HPA-1b platelets and αIIbβ3-transfected cell lines in comparison to the HPA-1a isoform. Shear-induced platelet adhesion onto immobilized immobilized fibrinogen was examined using an established model simulating arterial flow conditions. Prior to perfusion, platelets were fluorescently tagged, subsequently visualized by confocal LS microscopy, and quantified by digital imaging.

At shear rates of 1500 sec^{-1}, adhesion activity and thrombus formation of HPA-1b (Pro33) platelets were increased compared with HPA-1a (Leu33) platelets ($p<0.01$). Upon expression of both HPA-1 isoforms in CHO or HEK293 cells transfected with αIIbβ3, the HPA-1b (Pro33) variant displayed a higher adhesion stability than HPA-1a when exposed to increasing shear rates up to 1250 sec^{-1} ($p<0.05$). Analysis of Src, a tyrosine kinase constitutively associated with αIIbβ3, revealed that the specific activation of the phosphotyrosine motif at residue 418 (pSrc Y-418) was higher in adherent HPA-1b (Pro33) than HPA-1a (Leu33) platelets ($p<0.01$). Moreover, pSrc Y-418 in HPA-1b (Pro33) platelets reacted more sensitively upon incubation with Mn^{2+} than in HPA-1a (Leu33) platelets. Thus, HPA-1b (Pro33) platelets are characterized by (i) increased adhesion activity, (ii) increased thrombus stability, and (iii) increased outside-in signaling corresponding to their prothrombotic phenotype.

Recently, we have explored allosteric changes in the cytoplasmic tails of αIIbβ3 using fluorescence resonance energy transfer (FRET) and documented that quantitative differences between both isoforms are related to distinct conformational changes in their C-terminal domains upon receptor activation as a consequence of inside-out or outside-in signaling. These observations are in agreement with the contention that the HPA-1 polymorphism of αIIbβ3 may have a major impact on platelet adhesion, aggregation and thrombus formation under pathological conditions.

Ort: Hörsaal 6A
Zeit: Donnerstag, 31. Juni 2012, 17:00 Uhr c.t.
Gäste sind herzlich willkommen.