THE PLATELET SOCIETY

Platelet Society Meeting

Manchester

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Abstracts

THE PLATELET SOCIETY

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OC1

Platelets of ageing mice undermine lung endothelial barrier: The role of transforming growth factor β1 and SerpinA3K

Ebrahim H. Maleki[1,2], Marta Stojak[1], Anna Kurpinska[1], Anna Gdula[1], Agnieszka Kij[1], Marek Grosicki[1], Kamila Wojnar-Lason[1], Stefan Chlopicki[1,3], Marta Smęda[1]

[1]Jagiellonian University, Krakow, Poland. [2]Jagiellonian University Doctoral School of Exact and Natural Sciences, Krakow, Poland. [3]Jagiellonian University Medical College, Krakow, Poland

Abstract

Ageing leads to the progressive decline of endothelial function, alters the phenotype of circulating platelets, and undermines lung endothelial barrier. Platelets maintain the lungs' endothelial barrier integrity but simultaneously represent the major source of transforming growth factor β (mainly the TGFβ1 isoform) that can disrupt it. Using murine model, in this study we aimed to characterise the effects of ageing on platelet phenotype and platelet-dependent protection of the lung microvascular endothelial barrier integrity formed by primary lung microvascular endothelial cells (mLMVECs) in an *ex vivo* setting. We verified TGFβ1 released from age-altered platelets as the major factor that disrupts the integrity of the barrier formed by mLMVECs. By analysing the function of platelet-released individual proteins we identified SerpinA3K (S3K) as the platelet-derived protein that, acting as the serine protease inhibitor, inhibits the barrier-disruptive function of platelet-derived TGFβ1 and preserves the level of the tight junction protein claudin-5. These findings highlight that age-dependent changes in the platelet phenotype contribute to the alterations in the lung microvascular endothelial barrier integrity and identify platelet-derived S3K as a key determinant of the lung microvascular endothelial barrier response to platelet-derived TGFβ1.

OC2

Genome-wide assessment of mouse genes linked to hemostasis and platelet multi-omics to identify novel human bleeding disorders

Jingnan Huang[1], Federrico Marini[2], Fiorella Solari[3], Frauke Swieringa[4], Bas de Laat[5], Ilaria De Simone[4], Luigi Grassi[6], Xiang Gui[4], Kunpeng Li[1], Elizabeth Middleton[7], Neil Morgan[8], Isabella Provenzale[9], Carina Santos[10], Saskia Schols[11], Sarah Westbury[12], Albert Sickmann[3], Matthew Rondina[7], Wolfram Ruf[10], Mattia Frontini[13], Johan Heemskerk[4]

[1]Dept. of Nephrology, The First Affiliated Hospital, Southern University of Science and Technology, Shenzhen, China. [2]Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), Johannes Gutenberg-University Mainz, Mainz, Germany. [3]Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V, Dortmund, Germany. [4]Synapse Research Institute Maastricht, Maastricht, Netherlands. [5]Synapse Research Institute Maastrich, Maastricht, Netherlands. [6]National Health Service Blood and Transplant (NHSBT), Cambridge Biomedical Campus, Cambridge, United Kingdom. [7]Molecular Medicine Program, University of Utah, Utah, USA. [8]Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom. [9]Dept. of Biochemistry, Maastricht University, Maastricht, Netherlands. [10]Center for Thrombosis and Hemostasis (CTH), University Medical Center, Johannes Gutenberg-University Mainz, Mainz, Germany. [11]Dept. of Hematology, Radboud university medical center, Nijmegen, Netherlands. [12]Dept. of Haematology, Bristol Medical School, Bristol University, Bristol, United Kingdom. [13]Institute of Biomedical & Clinical Science, College of Medicine and Health, University of Exeter Medical School, Exeter, United Kingdom

Abstract

The hemostatic process relies on platelet and coagulation activation with additional orles of red blood cells and the vessel wall. By systematic screening of databases for gene-linked information on hemostasis, we collected phenotypic profiles of 3,406 orthologous human and mouse genes for bleeding, arterial thrombosis, thrombophilia, platelet, erythrocyte or coagulation traits. Listing showed that modifications in 254 mouse genes led to increased bleeding combined with platelet dysfunction or thrombocytopenia, on top of the 145 human orthologs which are already registered for bleeding association after panel sequencing. Additionally, 137 mouse genes contributed to arterial thrombosis without bleeding phenotype. To further establish the roles of platelets in hemostasis, we integrated multiple genome-wide proteomes and RNA-seq transcriptomes from healthy subjects and C57BL/6 mice. This provided reference levels for 54,255 (19,288) transcripts and 6,379 (4,563) proteins in human (mouse) platelets. Orthologous protein-encoding transcripts overlapped with R=0.75, while orthologous platelet proteins correlated with R=0.87. Comparison with phenotypic analysis revealed: (i) overall high similarity in human and mouse platelet composition and function; (ii) presence of transcripts in either platelets of most 3,406 phenotyped genes; (iii) preponderance of syndromic platelet-expressed genes; (iv) limited overlap with genes from genome-wide association studies. For 41 mouse genes among which receptors, signaling proteins and transcription regulators (ERG, GATA2, IKZF6, MEIS1, MRTFA, NFE2, TAL1), we confirmed a novel linkage with human platelet function or count. This inter-species comparison can serve as a valuable resource for researchers and clinicians studying the genetics of blood-borne hemostasis and thrombosis.

OC3

Peptide Supercharged Platelets Improve Cardiac Outcomes Post-Myocardial Infarction

Jonathan A. Furniss[1], Yong Li[1], Samantha Mason[2], Alexi Crosby[2], Nathalie Tarassova[1], Cedric Ghevaert[2], Alastair W. Poole[1]
[1]University of Bristol, Bristol, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

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OC4

TRIPLE Score: GPVI and CD36 Expression Predict a Prothrombotic Platelet Function Phenotype

Alexander Bye[1], Neline Kriek[1], Carly Kempster[1], Joanne Dunster[1], Joanne Mitchell[2], Tanya Sage[1], Suzannah Rawlings[1], Maria Diaz Alonso[1], Valentina Shpakova[1], Leanne Dymott[3], Sharon Mark[3], Mark Brunton[3], Joana Batista[4], Harriet McKinney[4], Patrick Thomas[4], Kate Downes[4], Amanda Unsworth[5], Neil Ruparelia[3], Charlie Mckenna[3], Chris Jones[1], Jonathan Gibbins[1]

[1]University of Reading, Reading, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom. [3]Royal Berkshire Hospital, Reading, United Kingdom. [4]University of Cambridge, Cambridge, United Kingdom. [5]University of Leeds, Leeds, United Kingdom

Abstract

Background: Platelet reactivity is a risk factor for cardiovascular events, but the mechanisms of platelet function variance are incompletely understood. This study investigated platelet biomarkers that determine platelet reactivity and thrombus growth that can guide use and development of antiplatelet agents.

Methods: Flow cytometric analysis of platelet function variables associated with *in vitro* thrombus formation were investigated in healthy subjects using linear regression and stepwise linear regression. Biomarkers associated with increased platelet function and *in vitro* thrombus volume were validated in a cohort of cardiology patients.

Results: Sensitivity to CRP-XL correlated with *in vitro* thrombus development ($r=0.622$, $p<0.01$) among healthy subjects (n=23). In a larger second cohort of healthy subjects (n=456), stepwise linear regression identified a three-parameter model based on platelet surface expression of CD36 and GPVI and subject age able to predict sensitivity to CRP-XL ($r=0.24$, MAE=0.27). Stratification of cardiology patients (n=252) using an algorithm based on these three parameters identified a subgroup of patients with high sensitivity to CRP-XL ($p<0.0001$) and predicted for increased *in vitro* thrombus formation ($p=0.031$).

Conclusions: Sensitivity of the GPVI response is a key determinant of thrombus formation which is in turn dependent on expression of platelet surface GPVI and CD36, as well as the age of the subject. Stratification of patients using these three parameters identifies a subgroup of patients with highly sensitivity GPVI responses and a prothrombotic platelet phenotype. Targeting GPVI and CD36 receptors may provide a more effective antiplatelet strategy for this subset of patients.

OC5

Affimer reagents targeting fibrinogen αC-region modulate fibrin clot contractability

Julia Sandrin Gauer, Helen McPherson, Maria Anastasiadi, Rui-Gang Xu, Cedric Duval, Christian Tiede, Fraser Macrae, Ramzi Ajjan, Helen Philippou, Darren Tomlinson, Robert Ariens

University of Leeds, Leeds, United Kingdom

Abstract

Background: We recently showed that the fibrinogen αC-region plays a critical role in GPVI-fibrin(ogen) binding and fibrin clotting. We thus hypothesise that targeting fibrinogen αC-region is an attractive target to reduce abnormal clot formation during thrombosis.

Aim: To determine the effects of fibrinogen αC-region targeting Affimers on platelet/clot interactions.

Methods: We developed 5 Affimers (αC-1,αC-5,αC-8,αC-20&αC-45) that selectively bind fibrinogen αC-region with high affinity. Affimer scaffold was included as control. *In-vivo* murine tail-vein injection followed by western-blotting was performed to determine half-life of Affimers in circulation. Clot/platelet contractability was investigated using whole blood and isolated platelets. Clot polymerisation/lysis profile and fibre density were performed by turbidity and confocal microscopy, respectively.

Results: In the presence of aC-8, whole-blood clot weight was reduced (41±5% with 25mM; $p<0.05$). Conversely, aC-5&aC-45 caused an increase in clot weight (41±6%&24±6% with 25mM&10mM, respectively; $p<0.5$). Isolated platelet retraction was also significantly slowed in the presence of aC-5. No changes were observed in platelet incorporation in the presence of any Affimer. Affimers αC-8 and αC-20 caused reduced turbidity maximum absorbance, accelerated clot lysis time (2.6&1.5-fold, respectively) and denser clot network compared to scaffold. I*n-vivo* experiments show that all αC-targeting Affimers remain in circulation for minimally one hour.

Summary/conclusions: Our data demonstrate that targeting the fibrinogen αC-region with Affimers is an attractive option to modulate platelet/clot interactions. The molecular and cellular mechanisms underpinning this, and effect of these reagents on thrombosis development *in-vivo* require further investigation.

OC6

Targeted BTK Degradation in Platelets by PROTACs: Preclinical Evidence

Carl May, Justin Trory, Hetty Walker, Yong Li, Connor Webb, Jay Tromans, Jordan Vautrinot, Ingeborg Hers

University of Bristol, Bristol, United Kingdom

Abstract

Introduction:

Despite advances in anti-thrombotic therapy, bleeding complications remain a critical challenge. Bruton's tyrosine kinase (BTK) is a promising platelet target, but conventional inhibitors frequently cause off-target effects. PROTAC-mediated BTK degradation offers a revolutionary approach, leveraging targeted protein degradation for precision therapy. NX-5948 and NX-2127, currently yielding exciting results in clinical trials for advanced B-cell malignancies, present unprecedented opportunities to explore this novel strategy in platelets.

Methods:

NX-5948 and NX-2127 were evaluated for BTK degradation efficiency and selectivity in human platelets. Off-target effects on TEC kinase and cooperativity in ternary complex formation were assessed. Given its superior potency and positive cooperativity, NX-5948 was selected for *in vivo* studies. Mice were treated with NX-5948, and platelet BTK levels were measured.

Results:

Both PROTACs achieved >95% BTK degradation in human platelets at low concentrations, sparing TEC. NX-5948 demonstrated robust positive cooperativity, enhancing ternary complex formation and protein degradation. *In vivo* administration of NX-5948 in mice resulted in significant BTK degradation in platelets.

Conclusion:

NX-5948 and NX-2127 are transformative agents, already showing clinical promise in trials for refractory B-cell malignancies, where NX-2127 has demonstrated durable complete responses. The successful degradation of BTK in platelets introduces a promising strategy for thrombosis prevention without compromising haemostasis. These findings mark a significant leap forward, with NX-5948 emerging as a frontrunner in redefining anti-thrombotic therapy.

OC7

PlateletBase: A Comprehensive Knowledgebase for Platelet Research and Disease Insights

Huaichao Luo

CHENGDU, CHENGDU, China

Abstract

Platelets are vital in many pathophysiological processes, yet there is a lack of a comprehensive resource dedicated specifically to platelet research. To fill this gap, we have developed PlateletBase, a knowledge base aimed at enhancing the understanding and study of platelets and related diseases. Our team retrieved information from various public databases, specifically extracting and analyzing RNA-seq data from 3,711 samples across 41 different conditions available on NCBI. PlateletBase offers six analytical and visualization tools, enabling users to perform gene similarity analysis, pair correlation, multi-correlation, expression ranking, clinical information association, and gene annotation for platelets. The current version of PlateletBase includes 10,278 genomic entries, 31,758 transcriptomic entries, 4,869 proteomic entries, 2,614 omics knowledge entries, 1,833 drugs, 97 platelet resources, 438 diseases/traits, and six analysis modules. Each entry has been carefully curated and supported by experimental evidence. Additionally, PlateletBase features a user-friendly interface designed for efficient querying, manipulation, browsing, visualization, and analysis of detailed platelet protein and gene information. Case study results, such as those from gray platelet syndrome and angina pectoris, demonstrate that this tool can aid in identifying diagnostic biomarkers and exploring disease mechanisms, significantly advancing research in platelet functionality and its applications. PlateletBase is accessible at http://plateletbase.clinlabomics.org.cn/.

OC8

The inhibitory effects of prostacyclin on platelet activation is lost in a hypoxic environment

Sophie Leonard, Zoe Booth, Monica Arman, Giordano Pula, Simon Calaminus

Hull York Medical School, Hull, United Kingdom

Abstract

Tissue hypoxia occurs in numerous pulmonary and cardiovascular conditions. These conditions like atherosclerosis, myocardial infarction, sleep apnoea and chronic obstructive pulmonary disease (COPD) are often associated with increased platelet activity or hyperreactivity. We propose that the hypoxic environment drives a hyperreactive platelet phenotype via the attenuation of the PGI_2/cAMP/PKA signalling pathway. Initially, we sought to investigate PGI_2 function by examining $pVASP^{ser157}$ and pPKA activity over a period of 20 minutes following treatment with PGI_2 (2nM and 10nM) at 1% and 21% O_2. We identified a clear reduction followed by complete loss of PKA activity at 1% O_2 after 20 minutes whilst, at 21% O_2 activity remained strong. Seeking to marry the signalling data with platelet activation, we undertook whole blood flow cytometry. Platelets were treated with PGI_2 (2nM and 10nM) before stimulation with ADP (10nM) and CRP (5µg/ml). In alignment with the PKA activity analysis, PGI_2 had little inhibitory effect on platelets kept in hypoxia while in normoxia, PGI_2 treatment inhibited platelet activation as expected. To further investigate the cAMP/PKA pathway we treated platelets with forskolin (10µM), milrinone (10µM) and iloprost (10nM) prior to ADP stimulation. A reduction in CD63, P-Selectin and PAC-1 MFI was observed in samples treated with each inhibitor in both hypoxia and normoxia, indicating a fully functional cAMP/PKA signalling pathway. We therefore propose that lack of response to PGI_2 in hypoxia is a result of two potential factors; elevated breakdown into 6-keto-PGFIα or a change in IP receptor expression.

OC9

Multiparameter flow cytometry allows the study of platelet hyposensitivity to PGI2 in coronary artery disease

Tudor Dimofte[1], Matthew Hindle[2], Amanda Unsworth[1], Stephen Wheatcroft[1], Mark Kearney[1], Khalid Naseem[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Leeds Beckett University, Leeds, United Kingdom

Abstract

Platelets play a central role in haemostasis, but they contribute to pathological inflammation and thrombosis when hyperactive. Platelet hyperactivity, the imbalance between platelet activation and inhibition, is key in coronary artery disease (CAD). Preliminary data suggests platelet from CAD patients are hyposensitive to the endogenous inhibitor PGI_2. Therefore, we sought to develop a novel multiparameter flow cytometry assay which allows for simultaneous assessment of platelet function and signalling, more precisely on the relationship between platelet activation and cAMP signalling. The work focussed on surface P-selectin, an activation-dependent marker that facilitates heterotypic cell interactions, and phospho-vasodilator-stimulated phosphoprotein (pVASP), a marker of intracellular cAMP signalling downstream of the platelet inhibitor PGI_2. Treatment of blood with a submaximal concentration of the synthetic, homonymous PAR1 agonist produced a substantial increase in P-selectin expression (10-fold) with little increase in pVASP levels. In contrast, treatment with a submaximal concentration of the endogenous platelet inhibitor PGI_2 produced a 10-fold increase in pVASP with no change in P-selectin expression. We then treated blood with a combination of PAR1 and PGI_2 and observed no change in P-selectin expression and a 10-fold increase in pVASP. This approach allowed us to demonstrate that the inhibition of P-selectin expression, and therefore platelet activation, was directly proportional to the strength of cAMP signalling. This multiplexed assay is the first example of examination of intracellular signalling and its link to functional changes in platelets in the complex milieu of whole blood and will facilitate further investigations into platelet hyposensitivity to endogenous inhibition in CAD.

OC10

Expansion microscopy allows quantitative characterisation of structural organisation of platelet aggregates

Emma Faulkner[1], Jeremy Pike[1], Evie Garlick[1], Robert Neely[1], Iain Styles[2], Stephen Watson[1], Natalie Poulter[1], Steve Thomas[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Belfast, Belfast, United Kingdom

Abstract

Our understanding of the structure of platelet aggregates and thrombi has been enhanced by the application of microscopy approaches. This has revealed important details of how the thrombi differ in haemostatic versus pathogenic conditions. However, current fluorescence microscopy approaches are not amenable to detailed volumetric imaging of platelet aggregate structures. This is largely due to the small size of individual platelets and the tight packing of platelets within aggregates, resulting in optical opacity.

Here we demonstrate that expansion microscopy, a super-resolution microscopy technique, reveals information about the structure of platelet aggregates and the intracellular distribution of proteins in individual platelets within the aggregate. We produced volumetric images at nanoscale resolution of >700 platelet aggregates under normal conditions and following cytoskeletal disruption. These have been stained for different proteins, including cytoskeletal and membrane components. Following imaging, we have applied custom image analysis workflows to extract quantitative descriptions of platelet numbers, volumes and morphology within entire platelet aggregates. Additionally, we quantitatively describe subcellular organisation of F-actin and the effect of platelet position in the aggregate on these parameters. By comparing these measurements following treatment with the actin inhibitors, cytochalasin D and latrunculin A, we can robustly detect structural disruptions in platelet aggregates. Together these data provide a workflow to qualitatively and quantitatively describe the architecture of platelet aggregates at a range of scales.

OC11

INVESTIGATING THE FEASIBILITY AND VALIDITY OF USING HUMAN PLACENTAL VESSELS FOR EX-VIVO MODELS OF THROMBOSIS AND HAEMOSTASIS.

Azziza Zaabalawi[1], Paul Brownbill[2], May Azzawi[1], Sarah Jones[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]University of Manchester, Manchester, United Kingdom

Abstract

Background: The study of thrombosis and haemostasis is dependent upon in-vivo models, which use large numbers of animals, and are limited by significant species differences. Human placentas represent a plentiful and underutilised source of human vessels, usually discarded following delivery. The study aim was to assess the feasibility and validity of using human placental vessels to develop novel ex-vivo models of thrombosis and haemostasis. **Methods**: Placental arteries were isolated from healthy pregnancies and cold-stored (3h,6h or 21h) or cryopreserved. Endothelial-dependent and independent dilation were assessed using organ-bath following pre-constriction with U46619 (10^{-6}M), and endothelial integrity verified using immunofluorescence. Haemostasis was assessed ex-vivo in placental vessels perfused in-situ with donor or autologous blood, by measuring bleeding time following defined puncture wounds (200µm,300µm,600µm) in the presence of Rivaroxaban, Glenzocimab, Vorapaxar or Cangrelor. Clot composition was analysed using SEM. **Results**: Endothelial integrity and endothelial-dependent and -independent dilation was preserved following 3h,6h and 21h cold-storage, and after cryopreservation. U46619-mediated constriction was maintained in all conditions. Interestingly, nitric oxide-dependent dilation was increased in vessels cold-stored for 6h and 21h compared to 3h ($P<0.05$), and was accompanied by significantly lower ROS levels ($P<0.05$). Autologous and donor blood was successfully perfused through intact vessels without spontaneous clotting. Needlestick vessel injury resulted in reproducible size-dependent haemostatic responses ($P<0.05$). Rivaroxaban, Glenzocimab and Vorapaxar significantly increased bleeding time ($P<0.05$) at all injury sizes, whilst Cangrelor only increased bleeding time with larger puncture wounds. **Conclusion**: Placental vessels offer a viable option to develop novel ex-vivo models of human thrombosis and haemostasis.

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OC12

Optimisation of Platelet Phenomic Analysis (PPA) to understand cardiovascular disease risk in people with HIV

Ami Vadgama[1], Akif Khawaja[1], Marta Boffito[2], Joanne Dunster[3], Jonathan Gibbins[3], Michael Emerson[1]

[1]Imperial College London, London, United Kingdom. [2]Chelsea and Westmister NHS Foundation Trust, London, United Kingdom. [3]University of Reading, Reading, United Kingdom

Abstract

Background: People with HIV (PWHIV) on effective antiretroviral treatment have increased risk of cardiovascular disease (CVD). The mechanisms behind this are currently unclear, although they may involve aberrant platelet function.

Aim: To optimise the Platelet Phenomic Analysis (PPA) platform – multicolour flow cytometry coupled with bioinformatics analysis – to characterise platelet function in people with HIV.

Methods: Platelet-rich plasma (PRP) from PWHIV was stimulated with a range of concentrations of ADP, CRP-XL, and TRAP-6. Flow cytometry was used to measure levels of fibrinogen binding, CD62P (P-selectin), and CD107a (LAMP-1). Median fluorescence intensity (MFI) was recorded, analysed using an ANOVA/mixed-effects analysis, and presented as mean MFI±SEM. Bioinformatics and machine learning approaches were also used.

Results: PPA provided detailed concentration responses to agonists. Differences were observed in the responses of platelets between patients prescribed different antiretroviral therapy combinations (integrase inhibitor-based: BIC/FTC/TAF, or non-nucleoside reverse transcriptase inhibitor-based: DOR/3TC/TDF). On stimulation with 0.3μM TRAP-6, P-selectin expression was significantly higher for PWHIV on BIC/FTC/TAF compared to DOR/3TC/TDF (877.0±11.6 vs. 748.8±24.1, n=4; p=0.02). Similarly, on stimulation with 30μM TRAP-6, CD107a expression was also raised in people on BIC/FTC/TAF compared to DOR/3TC/TDF (1065.0±38.0 vs. 744.3±22.3, n=4; p=0.008).

Conclusion: Using PPA, different platelet functional responses were detected between two different antiretroviral combinations. PPA provides a powerful, efficient, and reliable platform to explore the link between CVD and HIV. Studies are ongoing to characterise platelet function in PWHIV to better understand the underlying basis of increased CVD risk.

OC13

Engineering desialylation- and CD42 cleavage-resistant platelets to optimise storage and maintain lifespan and function following transfusion.

Amie Waller[1], Alexi Crosby[1], Winnie Lau[1], Krishnaa Mahbubani[1], Kourosh Saeb-Parsy[1], Renhao Li[2], Cedric Ghevaert[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]First Affiliated Hospital of Zhejiang University School of Medicine, Zhejiang, China

Abstract

Transfusion of platelets to either prevent or treat bleeding and maintain haemostasis, has been an effective therapy especially in patients undergoing chemotherapy or major surgery. Whilst an established therapy, platelet transfusion does present several difficulties, for example potential bacterial contamination due to the need to store platelet units at room temperature to prevent cold storage-induced lesions that reduce function and survival in circulation. Here, we used CRISPR Cas9 editing of inducible pluripotent stem cells (iPSCs), to knock-out *Neu1* (the major sialidase present in megakaryocytes (MKs) and platelets), *GP1bα* (the mechanosensory domain of GP1bα activates ADAM17 which then cleaves CD42) and *HLA* (to generate universal recipient platelets). These engineered iPSCs stably express three transcription factors (GATA1, FLI1, TAL1) that were integrated into the AAVSI locus safe harbour, under a doxycycline inducible promoter. A flow cytometry panel was developed measuring desialylation, activation and annexin V binding in platelets derived from iPSC-MKs during storage, over time, at 4, 21 and 37^{0}C. We hypothesise that platelets from these single, double and triple knock-out lines could be stored at lower temperatures and for longer periods, thus reducing wastage and the risk of bacterial contamination. Furthermore, a human *ex-vivo* spleen model, using reconstituted circulating blood, spiked with labelled platelets, has been validated both replacing the need for an animal model of senescent platelet clearance and providing evidence of splenic mobilisation of platelet populations.

OC14

Thrombotic response and aspirin effect in women with pre-eclampsia compared to healthy pregnancy

Isha Sabnis[1], Heather M Judge[1], Georgia M Williamson[1], Hannah C McMellon[1], Robert F Storey[1], Victoria L Parker[2], William A E Parker[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom

Abstract

Background

Aspirin 150 mg once-daily (OD) reduces the risk of pre-term pre-eclampsia but with residual risk. Peak-trough variation in aspirin effect can be accentuated by increased platelet turnover. In this pilot study, we sought to characterise platelet function and related markers in women with pre-eclampsia or healthy pregnancy (HP), and effectiveness of aspirin 150 mg OD in those receiving it.

Methods

Participants with a diagnosis of pre-eclampsia (n=15, median gestation 36 weeks, 8 receiving aspirin) or gestation-matched HP (n=15) were recruited. Maximum platelet aggregation (maxPA) responses were assessed by light transmittance aggregometry, P-selectin expression by flow cytometry, serum thromboxane (TX)B_2 by ELISA, platelet turnover by mean platelet volume (MPV) and fibrin clot dynamics by acellular turbidimetry.

Results

Although aggregation responses were similar between aspirin-free women with pre-eclampsia and HP, pre-eclampsia was associated with higher P-selectin expression to 30mmol/L-adenosine diphosphate (81.4% vs. 66.9%, p=0.028) and higher MPV (9.8fL vs. 9.2fL, p=0.032). Of those with pre-eclampsia, aspirin was associated with reduced mean maxPA to arachidonic acid (AA) and serum TXB_2, but when checked between 12 and 24 hours after the last dose, 3/8 women receiving aspirin had AA-induced maximum aggregation >20% and 4/8 had serum TXB2 >10ng/mL, despite self-reported compliance.

Conclusions

There was evidence of increased platelet reactivity and turnover in pre-eclampsia compared to HP. Aspirin 150 mg OD significantly inhibited platelet cyclooxygenase-1, but there was evidence of suboptimal round-the-clock effect in a significant proportion of women with pre-eclampsia.

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OC15

Platelets drive the formation of a pre-metastatic lung microenvironment.

Harvey Roweth[1,2,3], Emily Clarke[2], Margaret Smith[2,3], Estelle Carminita[4,3], Isabelle Becker[4,3], Elisabeth Battinelli[2,3]

[1]University of Reading, Reading, United Kingdom. [2]Brigham and Women's Hospital, Boston, USA. [3]Harvard Medical School, Boston, USA. [4]Boston Children's Hospital, Boston, USA

Abstract

The lungs represent a common site for metastatic breast cancer. This organotropism is created by the primary tumour, which remodels the lung niche into an immunosuppressive and pro-inflammatory space before tumour cell colonisation. While platelets are known to promote metastasis through direct interactions with tumour cells, their roles in creating a "pre-metastatic" lung microenvironment remain unknown.

To model pre-metastatic lungs, 4T1 breast cancer cells were injected into the mammary fat pads of BALB/cJ mice, which developed early and then established lung metastases after 10 and 21 days, respectively. Increased neutrophil and macrophage populations were detected within lungs before metastatic outgrowth, implying the generation of a pre-metastatic niche, while intravital two-photon microscopy demonstrated that platelets were also sequestered within lungs prior to established metastases.

To test if platelets are active contributors to the pre-metastatic niche, 4T1 mammary tumours were generated in mice with normal ($Thpo^{+/+}$) or low ($Thpo^{-/-}$) platelet counts. While low platelet counts had no effect on mammary tumour growth, platelet deficiency led to fewer neutrophils and macrophages within pre-metastatic lungs, with an increased proportion of $CD4^{+}$ lymphocytes. Our data show that platelets are sequestered within lungs before overt metastasis and recruit pro-inflammatory myeloid-derived suppressor cells known to generate the pre-metastatic niche. Ongoing experiments utilise single-cell RNA sequencing to assess if platelet deficiency ($Thpo^{-/-}$) leads to abnormal gene expression within pre-metastatic lungs. Future work will test if platelets influence other pre-metastatic sites and explore whether anti-platelet medications can limit malignancy by regulating the (pre)metastatic niche.

OC16

Brain-Derived Neurotrophic Factor Enhances Megakaryocyte Maturation and Promotes Proplatelet Formation

Imane Boukhatem[1,2], Izabella Andrianova[3], Abigail Ajanel[3], Mélanie Welman[2], Robert Cambell[4], Marie Lordkipanidzé[1,2]

[1]Université de Montréal, Montreal, Canada. [2]Montreal Heart Institute, Montreal, Canada. [3]Washington University School of Medicine, St-Louis, USA. [4]Washington University School of Medicine, St-Louis, Canada

Abstract

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin with key functions in the central nervous system and an emerging role in cardiovascular homeostasis. Platelets serve as the primary peripheral reservoir of BDNF, containing concentrations up to 1000-fold higher than neurons. However, the origin of platelet BDNF remains unclear, though evidence suggests megakaryocytic synthesis. We investigated the megakaryocytic origin of BDNF in human $CD34^+$-derived megakaryocytes and its role in megakaryopoiesis. Western blotting, flow cytometry, and confocal microscopy confirmed BDNF and TrkB expression in megakaryocytes and proplatelet extensions. Treatment with BDNF (200 ng/ml) increased megakaryocyte ploidy and proplatelet formation, while TrkB inhibitors GNF5837 (1 µM) and Cyclotraxin B (10 µM) or a BDNF-neutralizing antibody (10 µg/ml) reduced both. CRISPR-Cas9-mediated BDNF knockdown further impaired maturation and proplatelet formation, reinforcing its critical role in thrombopoiesis.

We also examined BDNF release during megakaryopoiesis. Culture supernatants showed a significant increase in BDNF levels on day 14, coinciding with proplatelet formation and the terminal phase of differentiation. Intracellular BDNF was present at days 7 and 14, but its release occurred only at the final stage, indicating a regulated secretion process. Additionally, mature megakaryocytes rapidly internalized extracellular BDNF, suggesting active modulation of BDNF availability by megakaryocytes.

These findings establish BDNF as a key regulator of megakaryocyte maturation and platelet function, providing direct evidence for its megakaryocytic origin. This study also highlights the dynamic interplay between BDNF secretion and uptake, laying the foundation for future research into its role in hematopoietic processes.

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P01

p75 Neurotrophin Receptor Partly Mediates BDNF-Induced Platelet Aggregation Without Affecting Inflammation or Apoptosis

Samuel Fleury[1,2], Imane Boukhatem[1,2], Jessica Blais[1,2], Anne-Sophie Marceau[1,2], Yanitsa Petkova[1,2], Mélanie Welman[1], Bruce G. Allen[1,3], H. Uri Saragovi[4,5], Marie Lordkipanidzé[1,2]

[1]Montreal Heart Institute, Montréal, Canada. [2]Faculty of Pharmacy, Université de Montréal, Montréal, Canada. [3]Departments of Biochemistry and Molecular Medicine, Pharmacology and Physiology, and Medicine, Faculty of Medicine, Université de Montréal, Montréal, Canada. [4]Lady Davis Institute, Jewish General Hospital, Montréal, Canada. [5]Department of Pharmacology and Therapeutics, McGill University, Montréal, Canada

Abstract

Background. The brain-derived neurotrophic factor (BDNF) induces aggregation of human platelets through the tropomyosin receptor kinase B (TrkB). The p75 neurotrophin receptor ($p75^{NTR}$) also binds BDNF and its precursor proBDNF. In neurons, $p75^{NTR}$ regulates inflammation through the production of reactive oxygen species (ROS) and apoptosis, but its role in platelets remains elusive. This project aims to identify the biological function of $p75^{NTR}$ in platelets by investigating platelet aggregation, ROS production and apoptosis using $p75^{NTR}$ ligands and inhibitors.

Methods. Platelets from healthy volunteers, isolated and resuspended in Tyrode's buffer, were activated using BDNF in the presence or absence of $p75^{NTR}$ inhibitors THX-B and TAT-pep5. Activation of signaling pathways was evaluated by phospho-blots. Production of ROS was quantified by flow cytometry using H_2DCFDA. Apoptosis levels were measured by flow cytometry using Annexin V labeling and activated caspase 3/7 probe FAM-DEVD-FMK.

Results. Unlike BDNF, its precursor proBDNF failed to induce platelet aggregation. $p75^{NTR}$ inhibitors partly inhibited platelet aggregation to BDNF, in a pathway distinct from the classical BDNF-associated STAT3 phosphorylation. Neither BDNF nor proBDNF induced platelet ROS production *per se*, nor did they alter platelet ROS production induced by toll-like receptor 1 agonist Pam3CDK4. Similarly, neither BDNF nor proBDNF induced platelet apoptosis or modified that induced by ABT-737.

Conclusion. $p75^{NTR}$ does not mediate ROS production or apoptosis in human platelets. However, $p75^{NTR}$ supports BDNF-induced platelet aggregation, to a lesser extent than the canonical BDNF receptor, TrkB. Future studies should focus on megakaryocytes, as there is no obvious phenotype in platelets.

P02

Spleen Tyrosine Kinase Phosphorylation at Y317 Negatively Regulates Both ITAM and hemITAM-Mediated Signaling and Function in Platelets

Satya Kunapuli, Manal Elzoheiry, Carol Dangelmaier, Dhruv Vajipayajula, Monica Wright, Alexander Tsygankov

Sol Sherry Thrombosis Research Center, Temple University Lewis Katz school of Medicine, Philadelphia, USA

Abstract

Spleen tyrosine kinase (Syk) is expressed in a variety of hemopoietic cells and its phosphorylation regulates downstream signaling events in platelets upon stimulation of glycoprotein VI (GPVI) and C-type lectin-like receptor II-type (CLEC-2) as ITAM and hemITAM receptors, respectively. This study focuses on the role of a specific phosphorylation site, Tyrosine 317, located in the linker region that separates the amino-terminal, tandem pair of SH2 domains from the carboxyl-terminal catalytic domain, in the regulation of Syk function. The amino acid sequence surrounding phosphotyrosine 317 matches the consensus sequence for recognition by the phosphotyrosine-binding (PTB) domain of the protooncogene product, c-Cbl. To evaluate the function of this phosphorylation site, we generated mice expressing Syk Y317F with CRISPR-Cas9 technique. Platelets from homozygous mice and wild-type (WT) littermates showed enhancement of functional responses and signaling phosphorylation levels in Syk Y317F after activation with collagen-related peptide (CRP) and CLEC-2 crosslinking. This enhancement did not occur after stimulation of PAR-4 or purinergic receptors with AYPGKF and 2-MeSADP, respectively. CRP- or CLEC-2 mAb- induced signaling events, including LAT and PLCγ2, were enhanced in Syk Y317F platelets compared to platelets from wild type littermates. Besides the functional increase, the time to occlusion in the $FeCl_3$ injury model was increased in Syk Y317F mice compared to WT littermates. However, there was no significant difference in the tail bleeding times. These data reveal that Tyrosine 317 negatively regulates Syk signaling and function in mice platelets.

P03

Preliminary analysis of O2 dynamics in stored platelet concentrates

Dean Pym[1,2], Amanda Davies[1], Jessica Williams[1], Christine Saunders[2], Chloe George[2], Philip James[1]

[1]Cardiff Metropolitan university, Cardiff, United Kingdom. [2]Welsh Blood Service, Cardiff, United Kingdom

Abstract

Neonatal platelet transfusions are vital for managing bleeding risk in thrombocytopenic neonates. Due to their limited blood volume, adult platelet doses are divided into smaller containers to reduce circulatory overload and donor exposure. These small-volume platelet concentrates (PCs) are typically stored for up to 5 days. While agitation during storage is believed to enhance oxygen exchange by maintaining an O_2 gradient across the bag, recent studies indicate that agitation-induced shear forces may compromise platelet viability and function over time. This study aimed to determine whether O_2 availability is limited in PC storage when stored for neonatal and adult transfusion. PCs for neonatal or adult transfusion were stored at 22°C on a 60rpm agitator either at 21% O_2 concentration ($[O_2]$) and 5% $[O_2]$. Electron paramagnetic resonance oximetry was used to measure PC $[O_2]$, oxygen consumption rate (OCR), influx of O_2 (Jin), and total PC OCR of stored PCs. Fickian-diffusion modelling predicted O_2 distribution. Results indicated that adult PCs had ~1.2-fold higher Jin and ~4.6-fold higher total OCR than neonatal PCs. However, neonatal PCs demonstrated ~4-fold greater Jin relative to total OCR, resulting in higher O_2 availability, greater $[O_2]$, and better maintenance of O_2 distribution. Neonatal PCs tolerated reduced ambient O_2 levels (5%) without hypoxia, whereas adult PCs exhibited significant hypoxia. The study concluded that current storage guidelines do not limit O_2 availability. However, neonatal PCs outperform adult PCs in maintaining O_2 levels under both normal and reduced O_2 conditions, challenging the necessity of agitation for small-volume PCs.

P04

A case study of platelet function in critical illness with liver disease

Tyler Horn[1,2], Liza Keating[2,1], Matthew Frise[2,1], Jonathan Gibbins[1], Craig Hughes[1]

[1]University of Reading, Reading, United Kingdom. [2]Royal Berkshire Hospital, Reading, United Kingdom

Abstract

Critically ill patients with liver disease experience dysregulated haemostasis resulting in bleeding in some patients and thrombosis in others. Previous studies often focus on absolute platelet number; this however, does not always correlate with physiological outcomes. Outside of critical care platelet function has been shown as more important than absolute platelet number. However, platelet function has yet to be explored in critically ill patients with liver disease.

Here we describe an ICU patient who presented with Acute on Chronic Liver Failure on a background of alcoholism. Platelet function and receptor expression were measured on initial ICU admission and then again 48-hours later. Initially, the patient had suspected liver fibrosis, no prior evidence of cirrhosis and a suspected portal vein thrombosis. 48-hours post-admission the patient had experienced melena and therefore suspected upper gastrointestinal bleed.

A decrease in platelet number was observed between the two samples from 109 to 86 x10^9/L. Subsequent platelet function analysis by flow cytometry showed a decrease from sample 1 to sample 2 in integrin activation and P-Selectin exposure in response to ADP, CRP-XL, TRAP-6. Platelets at 48 hours post-admission exhibited decreased surface expression of GPIX and CD36 and decreased ability to form thrombin *in vitro* perfused at arterial flow conditions over collagen.

This case suggests that platelet count alone may not be responsible for the dysregulated haemostasis and bleeding state. This motivates further work to determine if changes in platelet biogenesis, receptor levels, and functional properties are associated with liver dysfunction and related complications.

P05

The rs1187325 Variant of the Gene Encoding Tyrosine Kinase B (TrkB) is Associated With Increased Platelet Sensitivity.

Jessica Blais[1,2], Imane Boukhatem[1,2], Samuel Fleury[1,2], Florence Lipp[1,2], Melanie Welman[1], Georges Jourdi[1,2,3,4], Marie Lordkipanidzé[1,2]

[1]Montreal Heart Institute, Montreal, Canada. [2]Faculty of pharmacy, University of Montreal, Montreal, Canada. [3]Université Paris Cité, Institut National de la Santé et de la Recherche Médicale, Innovative Therapies in Haemostasis, Paris, France. [4]Service d'Hématologie Biologique, Assistance Publique : Hôpitaux de Paris, Hôpital Lariboisière, Paris, France

Abstract

Introduction

Brain-Derived Neurotrophic Factor (BDNF) mediates its neuronal and platelet effects via activation of its receptor, TrkB. Multiple genetic variations are listed in the gene encoding TrkB. The rs1187325 (C>G) variant has been reported to be associated with increased TrkB expression in endothelial cells and with increased cardiovascular risk. The aim of this study is to determine the effect of the rs1187325 variation on platelet function.

Methodology

Participants homozygous for the reference allele (CC) or the variant allele (GG), matched by sex and age (±5 years), were recruited from the ICM Biobank. TrkB expression levels were measured by flow cytometry, and platelet response was determined by lumi-aggregometry.

Results

Recruitment and matching involved 7 women and 7 men homozygous for either the G allele or the reference C allele. The level of TrkB receptor expression in platelets was significantly higher in G allele carriers (79.1± 22.0%) compared to C allele carriers (69.4± 21.3%, p=0.04). Functionally, a difference in the collagen concentration-response curve (EC50CC=0.45 µg/ml vs. EC50GG=0.91 µg/ml) was observed, indicating an increased sensitivity to activation in platelets for the carrier of the C allele.

Conclusion

Carriers of the G allele show higher TrkB expression, while carriers of the C allele exhibit increased sensitivity to collagen-induced platelet activation. These results underscore the role of the neurotrophic system in hemostasis and thrombosis.

P06

Development of novel, small-scale blood sampling for platelet function analysis

Abhishek Sadhu, Tyler Horn, Hind Alosaimi, Chris Jones, Craig Hughes

University of Reading, Reading, United Kingdom

Abstract

Platelet function assays currently require venous blood collection, which presents challenges in populations without trained phlebotomists. Capillary sampling offers a minimally invasive alternative that facilitates point-of-care testing and expands accessibility. We have developed a novel micro-sampling methodology for humans and mice and evaluated it for platelet function analysis.

Blood was collected by venipuncture or our new finger-prick sampling method in humans and by cardiac puncture or modified tail vein sampling in mice. These methods use standard equipment, including lancet (humans) or scalpel (mouse), parafilm, and citrate anticoagulant. Prior to bleeding, the sampling site was warmed, and the first drop of blood was discarded to avoid tissue factor contamination. The subsequent blood drops were collected directly onto the parafilm and then gently mixed with anticoagulant.

Platelet activation was assessed by flow cytometry, measuring fibrinogen binding and P-selectin exposure in response to ADP, CRP-XL, TRAP-6 in humans, and fibrinogen binding in response to ADP, CRP-XL in mice. In humans, platelet sensitivity (EC_{50}) and capacity (maximal minus minimal response) showed no significant differences between capillary and venous samples, except for a higher EC_{50} of P-selectin exposure to CRP-XL in capillary samples (0.179 µg/mL), compared to venous samples (0.026 µg/mL). In mice, no significant differences were observed in EC_{50} or capacity in response to ADP or CRP-XL between cardiac puncture and our modified tail vein method.

Our new bleeding methods are viable alternatives to the commonly used methods, while improving accessibility and aligning with the ethical principles of NC3Rs for animal usage.

P07

Delineating Zinc Influx Mechanisms during Platelet Activation

Sahithi Kuravi[1], Niaz Ahmed[1], Kirk Taylor[2], Emily Capes[1], Alex Bye[2], Amanda Unsworth[3], Jonathan Gibbins[2], Nicholas Pugh[1]

[1]Anglia Ruskin University, Cambridge, United Kingdom. [2]University of Reading, Reading, United Kingdom. [3]University of Leeds, Leeds, United Kingdom

Abstract

Zinc (Zn^{2+}) is released by platelets during a haemostatic response to injury. Extracellular zinc ($[Zn^{2+}]_o$) initiates platelet activation following influx into the platelet cytosol. However, the mechanisms that permit Zn^{2+} influx are unknown. Fluctuations in intracellular zinc ($[Zn^{2+}]_i$) were measured in fluozin-3-loaded platelets using fluorometry and flow cytometry. Platelet activation was assessed using light transmission aggregometry. The detection of phosphoproteins was performed by Western blotting. $[Zn^{2+}]_o$ influx and subsequent platelet activation were abrogated by blocking the sodium/calcium exchanged, TRP channels, and ZIP7. Cation store depletion regulated Zn^{2+} influx. $[Zn^{2+}]_o$ stimulation resulted in the phosphorylation of PKC substates, MLC, and β3 integrin. Platelet activation via GPVI or Zn^{2+} resulted in ZIP7 phosphorylation in a casein kinase 2-dependent manner and initiated elevations of $[Zn^{2+}]_i$ that were sensitive to the inhibition of Orai1, ZIP7, or IP3R-mediated pathways. These data indicate that platelets detect and respond to changes in $[Zn^{2+}]_o$ via influx into the cytosol through TRP channels and the NCX exchanger. Platelet activation results in the externalization of ZIP7, which further regulates Zn^{2+} influx. Increases in $[Zn^{2+}]_i$ contribute to the activation of cation-dependent enzymes. Sensitivity of Zn^{2+} influx to thapsigargin indicates a store-operated pathway that we term store-operated Zn^{2+} entry (SOZE). These mechanisms may affect platelet behaviour during thrombosis and haemostasis.

P08

Endothelial BACE1 regulates platelet activity in haemostasis and thrombosis

Beth Webb, Lih Cheah, Mary McKay, Amanda MacCannell, Amanda Unsworth, Cedric Duval, Robert Ariens, Paul Meakin, Khalid Naseem

Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), Leeds, United Kingdom

Abstract

Background: Nitric oxide (NO) released from the endothelium regulates platelet activation and haemostasis via cyclic guanosine monophosphate (cGMP)-dependent protein kinase G (PKG) signalling. Reduced NO levels, which are associated with platelet hyperactivity in cardiovascular diseases may be influenced by elevated activity of Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1). Inhibition of BACE1 enhances endothelial nitric oxide synthase (eNOS) activity, increasing NO production and resulting in vasodilation and lowered blood pressure. However, the role of endothelial BACE1 in modulating platelet function remains unexplored.

Aims: This study investigates the influence of endothelial BACE1 activity on platelet activation, haemostasis, and thrombosis.

Methods: Phosphoflow cytometry, bleeding time, haemoglobin analysis, and *in vivo,* thrombosis assays were conducted in global and endothelial-specific BACE1 knockout (KO) mice, with and without a high-fat diet (HFD).

Results: Global BACE1-KO mice displayed normal thrombosis in response to ferric chloride-induced injury but showed significantly prolonged bleeding times compared to wild-type controls. Similar findings of increased bleeding time were observed in endothelial-specific BACE1-KO mice, with and without HFD. Phosphoflow analysis revealed elevated basal phosphorylation of platelet $VASP^{Ser239}$ in endothelial-specific BACE1-KO mice, consistent with enhanced cGMP signalling due to increased NO in circulation.

Conclusions: Loss of endothelial BACE1 increases eNOS activity, supporting NO production and attenuating platelet activation via cyclic nucleotide pathways. These findings reveal a novel regulatory mechanism linking endothelial BACE1 activity to platelet function, with potential implications for cardiovascular disease therapeutics.

P09

Investigating the role of UBE2N in platelet activation and function

Mugthalana Allet[1], Jon Jon Humphries[2], Khalid Naseem[1], Amanda Unsworth[1]

[1]Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), University of Leeds, Leeds, United Kingdom. [2]Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University., Manchester, United Kingdom

Abstract

Increased platelet activation can lead to thrombosis. Current anti-platelet therapies carry an increased risk of bleeding, making it essential to explore alternative approaches. Ubiquitination is a post-translational modification in which ubiquitin is attached to lysine residues on the target proteins via E1, E2, and E3 ubiquitin ligases.Previous studies have shown that the process of ubiquitination impacts platelet function, but the roles of ubiquitin modulators in platelet signalling remains poorly understood. It has been shown that deletion of UBE2N (E2 ligase) reduces platelet count, but its role in platelet function is unknown. UBE2N ubiquitinates proteins via the Lys63 linkage, linked to signalling events, suggesting UBE2N may play a broader role in platelet signalling.This research aims to investigate whether UBE2N, is involved in platelet signalling and function.

Using Western Blotting, we confirmed the presence of UBE2N in human platelets. Platelet aggregation assays were performed following treatment with pharmacological inhibitors NSC697923 and BAY11-7082 (0, 0.1, 1, 10 μM) . We observed inhibition of platelet aggregation responses to collagen and U46619 following treatment with both NSC697923 and BAY11-7082 but not thrombin. In addition, platelet adhesion and spreading were also found to be reduced by the UBE2N inhibitors compared to vehicle treated control.

Our results indicate that UBE2N positively regulates platelet function, potentially through signalling pathway shared by collagen, U46619 and fibrinogen. These findings identify novel anti-platelet activity of UBE2N inhibitors, suggesting its significant role in platelet activation and its potential as a future therapeutic target in thrombotic diseases.

P10

CHARACTERISTICS OF PHOSPHATIDYLINOSITOL-3,4,5-TRISPHOSPHATE (PIP_3) BINDING TO KINDLIN-3 IN HUMAN PLATELETS.

Kalwant Authi

King's College London, London, United Kingdom

Abstract

Kindlin-3 and Talin are essential for integrin activation in platelets, but how Kindlin-3 is activated is not well understood. Kindlin-3 contains a pleckstrin homology (PH) domain that potentially binds PIP_3 however high affinity binding has thus far not been demonstrated. Here PIP_3 binding to Kindlin-3 using PIP_3-beads is examined.

Fresh human platelets were lysed with NP40 (0.5%) and lysates were treated with PIP_3-beads in the presence or absence of reagents of interest and incubated for 45 min at 4C with shaking. The beads were isolated by centrifugation and washed 3 times before being analysed by western blotting.

PIP_3-beads potently and efficiently extracted the PH domain containing proteins RASA3 and BTK. No extraction was observed with control beads and extraction of both RASA3 and BTK was competed by adding exogenous DiC8-PIP_3 or IP_4 to the lysates. When Kindlin-3 was examined with PIP_3 -beads none was extracted. However when lower 0.2% NP40 was used there was faint detection of Kindlin-3 in PIP_3 extracts. Further addition of exogenous PIP_3 or IP_4 to the incubations enhanced Kindlin-3 extraction in contrast to competition of extraction of RASA3 or BTK. Stimulation of platelets with thrombin before lysis and incubation with PIP_3 led to an enhancement of PIP_3 binding. Incubation of reagents known to bind PH domains such as AKTI-IV (an inhibitor of AKT phosphorylation) and SC79 (a stimulator of AKT phosphorylation) also enhanced extraction of Kindlin-3.

In conclusion Kindlin-3 only binds PIP_3 after conformational changes to its PH domain.

P11

Regulation of platelet-neutrophil communication via connexin gap junctions

SALIHAH ALALYAN, Alice Pollitt, Jonathan M Gibbins

University of Reading, Reading, United Kingdom

Abstract

Background:

Platelets are recognised for playing roles beyond thrombosis and haemostasis and mediating inflammation by direct interactions with innate immune cells. Connexins are membrane proteins that form hexameric hemichannels, which dock to create gap junctions between platelets, allowing adjacent cells to communicate directly through intercellular signalling. Platelets express several connexin family members, including Cx37, Cx40 and Cx62. Notably, Cx37 and Cx40 are also expressed in neutrophils. Given the importance of platelet interactions with neutrophils, we evaluated the potential for platelet connexins to mediate neutrophil-platelet interactions and intercellular communication.

Methods and Results:

Platelet-neutrophil interactions were investigated using neutrophils stained with Fura-2 (ratiometric dye, 2 μM) or calcein AM (cytosolic dye,0.1μM). Neutrophils were mixed with washed platelets and stimulated with CRP-XL (1 μg/mL) or fMLP (1 μM). Intracellular calcium mobilisation was measured by fluorescence spectroscopy. CRP-XL stimulation of platelets induced calcium mobilisation in neutrophils, which was inhibited by carbenoxolone (Cbx (100μM), 35%), or connexin selective inhibitors ^{37,43}Gap27 (Cx37, (100 μg/mL), 21%) and ^{40}Gap27 (Cx40 (100 μg/mL), 16%) compared to vehicle or scrambled peptides. CRP-XL exposure to Fura-loaded neutrophils (without platelets) did not induce intracellular calcium mobilisation, whereas fMLP did. To assess fMLP-evoked connexin channel opening, calcein (0.62 kDa) efflux from neutrophils to platelets was analysed via confocal microscopy. Fluorescence diffusion was noted from donor neutrophils to platelets. This process was inhibited by Cbx, ^{37,43}Gap27, and ^{40}Gap27, confirming the involvement of connexins in neutrophil-platelet intercellular communication.

Conclusion:

These data indicate that connexin channel-mediated intercellular communication occurs between neutrophils and platelets.

P12

Characterising the Effects of Commonly Prescribed Antiretroviral Drugs on Platelets to Improve Understanding of Cardiovascular Risk in People with HIV

Tessa Siddall, Ami Vadgama, Mike Emerson

Imperial College London, London, United Kingdom

Abstract

Introduction:

Effective antiretroviral therapy has dramatically improved the life expectancy of people with HIV, but cardiovascular disease (CVD) risk is doubled, platelet-driven thrombotic events are more prevalent, and platelet activation is altered. Previous studies have shown off-target effects of antiretrovirals including nucleotide reverse transcriptase inhibitors (NRTIs) on platelet reactivity however, the impact of more modern HIV drugs, such as integrase strand transfer inhibitors (INSTIs), is unknown. We aimed to establish the effects of the INSTIs dolutegravir (DTG) and bictegravir (BIC) on platelet activation and the inhibition of activation to better understand their CVD risk profile.

Methods:

Platelets were isolated from individuals without HIV and platelet function was evaluated using 96-well plate light transmission aggregometry (LTA). Platelet-rich plasma (PRP) was isolated and incubated with INSTIs and drugs that mimic endothelial-mediated inhibition of activation. Platelet aggregation was measured in the presence of TRAP-6, ADP, and collagen.

Results:

Platelet activation was unaffected by acute administration of all antiretrovirals tested, but DTG augmented inhibition of aggregation by NONOate and Iloprost under some conditions. The effects of DTG were also significantly different to BIC. Observed effects were specific to the agonists ADP and TRAP-6 but were not seen with collagen-induced aggregation suggesting modulation of specific pathways.

Conclusions:

Our data currently provides preliminary evidence linking INSTIs to inhibition of activation. Further research using more physiologically relevant models is required. The implementation of microfluidic flow assays, animal studies, and patient investigations will enable novel insights into platelet-endothelial interactions relevant to CVD in people with HIV.

P13

Investigating the Effects of Indoxyl Sulphate on Thrombosis and Haemostasis.

Bismah Mateen[1], Philip Kalra[2,3], Amanda Unsworth[4], Sarah Jones[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Northern Care Alliance NHS Foundation Trust, Salford, United Kingdom. [3]University of Manchester, Manchester, United Kingdom. [4]University of Leeds, Leeds, United Kingdom

Abstract

Background: Chronic kidney disease (CKD) is a progressive disease affecting 800 million people worldwide and is associated with cardiovascular disease. Haemostasis is severely perturbed in these patients increasing the risk of both bleeding and thrombosis. The mechanisms that underpin these alterations however are not fully understood. Indoxyl sulphate (IS) is a protein-bound uremic toxin which progressively accumulates in CKD, as disease progresses.

Aim: The aim of this study was to determine whether altered IS levels associated with the different stages of CKD alter haemostasis.

Methods: Lumi-aggregometry was used to measure platelet aggregation and dense-granule secretion in platelet rich plasma (PRP). Impedance aggregometry was used to measure aggregation in whole blood and thrombus formation was measured in whole blood under arterial shear rates (1000-s). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured in platelet poor plasma. Calcium flux was measured in platelets loaded with fluorescent indicator Fura-2AM. All experiments were performed in the presence of IS (50-400µg/mL).

Results: IS alone had no effects on platelet function, with no aggregation, secretion, coagulation or calcium flux observed. Agonist-induced platelet aggregation was not enhanced or reduced by IS in PRP (n=5) or whole blood (n=4) and dense granule secretion also remained unaffected. Thrombus formation on immobilised collagen under flow was also not altered by the presence of IS (n=4).

Conclusion: IS does not directly alter platelet function or coagulation. More research is therefore required to understand the mechanisms that underpin increased risk of bleeding and thrombosis in CKD patients.

P14

Impact of the Prohibitin 1 rs2233667 variant on Megakaryocyte and Platelet Biology

Charlotte Aubin[1,2], Yanitsa Petkova[1,2], Mélanie Welman[1], Imane Boukhatem[1,2], Jessica Blais[1,2], Samuel Fleury[1,2], Georges Jourdi[1,2,3,4], Marie Lordkipanidzé[1,2]

[1]Research Center, Montreal Heart Institute, Montreal, Canada. [2]Faculty of Pharmacy, Université de Montréal, Montreal, Canada. [3]Université Paris Cité, INSERM, Therapeutic Optimization in Neuropharmacology, Paris, France. [4]Biological Hematology Department, Hôpital Lariboisière, Paris, France

Abstract

Background. Prohibitin regulates apoptosis, inflammation, and metabolism, acting as a protective or risk factor depending on expression, stimuli, and cell type. It regulates NGFR expression, which encodes for the $p75^{NTR}$ receptor, a binding partner of the Brain-Derived Neurotrophic Factor (BDNF) with a suspected role in platelet aggregation. This project investigates the impact of variant rs2233667 (G>T), located in the intron 6 of PHB1, on prohibitin and $p75^{NTR}$ expression in platelets and megakaryocytes, as well as platelet activity.

Methods. Coagulation profiles and blood counts of homozygous carriers of the variant allele (T/T, n=14) were compared to age- and sex-matched carriers of the reference allele (G/G, n=14). Flow cytometry was used to quantify biomarkers and proteins of interest in platelets and megakaryocytes. Platelet aggregation was assessed with light transmission aggregometry, while ROTEM was used to assess thromboelastic properties of clots.

Results. Prohibitin was absent in permeabilized platelets and megakaryocytes in carriers of the reference genome, but it was significantly overexpressed in variant carriers' megakaryocytes. Carriers of the variant also expressed significantly higher levels of $p75^{NTR}$ in their megakaryocytes, but not in platelets where $p75^{NTR}$ expression was significantly lower. Light transmission aggregometry showed reduced response to BDNF in variant carriers, but surprisingly increased sensitivity to TRAP. While general coagulation remained unaffected, thromboelastometry revealed reduced clot rigidity, stability, and fibrinolysis resistance in carriers.

Conclusion. Further studies are needed to determine PHB1 localization in megakaryocytes and platelets, to better understand its interaction with $p75^{NTR}$ expression in these cells and its functional consequences.

P15

Reported TMEM16F inhibitors do not block platelet phosphatidylserine exposure.

Ruby Baxter, Cameron Bicknell, Milena A. Malcharek, Matthew Harper

Department of Pharmacology, University of Cambridge, Cambrdge, United Kingdom

Abstract

Background: Pro-coagulant platelets expose phosphatidylserine (PS), promoting thrombin generation in thrombosis and haemostasis. TMEM16F is the 'scramblase' responsible for PS exposure. Inhibition of TMEM16F and therefore PS exposure could be a novel approach to prevent thrombosis. Although several drugs are reported to inhibit TMEM16F, there is limited evidence of their efficacy with platelets.

Aims: Assess the efficacy of reported TMEM16F inhibitors on platelet PS exposure.

Methods: Platelets were incubated with reported TMEM16F inhibitors before stimulation with thrombin and CRP-XL or A23187. PS exposure was measured with Annexin V (AnV) and mitochondrial membrane potential was measured with TMRM, via flow cytometry.

Results: Abamectin and niclosamide reduced the percentage of AnV-positive platelets following thrombin and CRP-XL stimulation but not following A23187. The MFI of the AnV-positive population was unchanged suggesting that there was no reduction in the extent of PS on the platelet surface. Abamectin reduced degranulation and αIIbβ3 integrin activation with stimulation showing inhibition of platelet activation which could account for the reduction in the percentage AnV-positive platelets. Niclosamide incubation resulted in loss of TMRM fluorescence in the absence of stimulation to a similar extent as CCCP (a mitochondrial uncoupler). CCCP similarly reduced the percentage of AnV-positive platelets with thrombin and CRP-XL stimulation, suggesting that niclosamide could also be a mitochondrial uncoupler.

Conclusions: Reported TMEM16F inhibitors do not selectively inhibit platelet PS exposure in repsonse to stimulation. Abamectin and niclosamide showed a reduction in AnV-positive platelets with thrombin and CRP-XL treatment, but this can be attributed to off-target effects.

P16

Platelet mediated immunometabolic reprogramming of peripheral blood mononuclear cells (PBMCs).

Emily J.M. Horner, Ramzi A. Ajjan, Amanda J. Unsworth, Khalid M. Naseem

University of Leeds, Leeds, United Kingdom

Abstract

Cardiovascular diseases (CVDs) remain a leading cause of global morbidity and mortality, which is enhanced when coupled to the comorbidity of Type 2 Diabetes (T2D). The molecular and cellular mechanisms through which T2D drives accelerated CVDs remain unclear. In both disorders, thrombo-inflammation, defined as a feedback loop of low-grade inflammation and thrombosis, has emerged as a potential pathologic mechanism. Underlying the inflammation are dysfunctional immune cells, which shift to a proinflammatory phenotype, with alterations in metabolic fuel utilisation and the eventual loss of mitochondrial function as potential drivers. Previous studies have established that immune cells can be “educated” by platelets, to enhance interleukin-1b production. The mechanisms driving these changes remain unclear.

The hypothesis underpinning this project is that platelets act as immune rheostats, which drive metabolic reprogramming of peripheral blood mononuclear cells (PBMCs), enhancing their conversion to a proinflammatory phenotype, which is exacerbated in T2D.

Platelet releasates, prepared from the secreted granule contents of thrombin-stimulated platelets, were quantified. Investigation into the effect of platelet releasates on PBMC metabolic pathways, using Seahorse Assays, demonstrated an immediate, significant increase in glycolysis, measured using extracellular acidification rate (ECAR), following the addition of platelet releasates, compared to basal PBMCs or thrombin treatment. No significant differences were observed in oxygen consumption rate (OCR), measuring oxidative phosphorylation.

This preliminary observation indicates platelet-driven metabolic reprogramming of PBMCs. Further studies using inhibitors of the four key glycolytic steps, alongside investigation of inflammatory cytokine synthesis and production will be completed to determine how platelets alter PBMC immunometabolic signalling pathways.

P17

PPAnalysis Identifies Complex Association between Platelet Phenotype and Obesity

Joanne Dunster[1], Neline Kriek[1], Alexander Bye[1], Tanya Sage[1], Maria Diaz Alonso[1], Valentina Shpakova[1], chris Jones[1], Amanda Unsworth[2], Jonathan Gibbins[1]

[1]University of Reading, Reading, United Kingdom. [2]University of Leeds, Leeds, United Kingdom

Abstract

Background

The effects of obesity on platelets are complex and inconsistent across studies. Some research links obesity to increased platelet count and mean platelet volume (MPV), while others report no change or even a decrease. Limited evidence suggests heightened platelet aggregation and resistance to inhibitors in obesity, alongside GPVI pathway dysregulation. These discrepancies highlight the need to understand the impact of obesity on platelet function and cardiovascular disease risk.

Aims

To characterise platelet function phenotypes within the population and their relationship with obesity.

Methods

Platelet-rich plasma (PRP) from 150 donors was analysed using PPAnalysis (high-throughput 96-well plate flow cytometry) to measure platelet activation (P-selectin exposure, fibrinogen binding) in response to three agonists at multiple concentrations. The associated open-source R package extracted Sensitivity (EC50) and Capacity (Emax) metrics. Unsupervised clustering categorised these 12 platelet function measures, which were then assessed against donor characteristics.

Results

Sensitivity and Capacity were independent variables, with limited correlation between P-selectin exposure and fibrinogen binding. Correlations with donor characteristics varied by metric; for example, higher BMI was linked to reduced Capacity, with gender-specific differences. Machine learning identified eight distinct platelet function subpopulations displaying combinations of high to low Capacity and Sensitivity, with statistically significant correlations ($p<0.001$) between BMI and platelet function that are associated with high cholesterol and HOMA-IR.

Summary

High-dimensional assay data and machine learning identified a platelet function phenotype linked to high BMI and other cardiovascular risk factors. This functional analysis could support patient stratification, advancing precision anti-platelet therapy.

P18

Platelet-mediated modulation of plasmin generation

Kieran Pocknell[1], Ilaria De Simone[2], Samia Tufaha[1], Bas de Laat[2], Claire S Whyte[1], Dana Huskens[2], Nicola J Mutch[1]

[1]Insitute of Medical Sciences, School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Aberdeen, United Kingdom. [2]Synapse Research Institute, Maastricht, Netherlands

Abstract

Background

Platelets harbour fibrinolytic factors including plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI) and plasminogen which are exposed upon stimulation. A high throughput plasmin generation (PG) assay has been developed but the sensitivity of this assay to platelets is unknown.

Aim

To investigate the impact of platelet-derived fibrinolytic factors on a novel PG assay.

Method

Matched healthy donor platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were clotted using tissue factor (1 pM), and $CaCl_2$ (16.7 mM). Tissue plasminogen activator (tPA; 20 nM) mediated PG was quantified by fluorogenic substrate. PRP was stimulated with CRP-XL (1 µg/ml) and (TRAP-6, 30 µM). In some experiments, thrombomodulin (TM; 20 nM), anti-PAI-1(5 µg/ml), or carboxypeptidase inhibitor (CPI; 25 µg/ml) were included.

Results

Unstimulated PRP reduced the plasmin peak (49.2±2.8 nM) compared to matched PPP (69.1±7.4 nM). In contrast, stimulated PRP enhanced the plasmin peak (49.2±2.8 vs. 63.7±4.2 nM) and shortened the lag time (4.6±0.06 vs. 3.4±0.1 min) compared to unstimulated PRP. Neutralising PAI-1 had no impact on PG. PG was downregulated by TM to a similar degree in PPP and PRP (11.3±2.7 and 12.8±3.2 nM) and was reversed by CPI which inhibits activated TAFI.

Conclusion

The PG assay was not sensitive to platelet-derived PAI-1, and inclusion of platelets did not augment the inhibitory effect of TM-mediated TAFI activation over that observed in PPP. Interestingly, strong platelet stimulation augmented PG, potentially by facilitating assembly of profibrinolytic proteins on the activated membrane.

P19

Supercharged platelets as a novel therapy for reducing blood loss

Alexi Crosby[1], Holly Foster[1], Winnie Lau[1], Solomon Shepherd[1], Amanda Evans[1], Amie Waller[1], Souradip Mookerjee[1], Lily Du[2], David Wilcox[2], Cedric Ghevaert[1]

[1]Cambridge University, Cambridge, United Kingdom. [2]Medical College of Wisconsin, Milwaukee, USA

Abstract

Background

Each year 280,000 units of donor-derived platelets are transfused in the UK. We currently rely on allogenic donation, which has major drawbacks. Concentrated coagulation factors, including recombinant FVIIa (rFVIIa), can be administered. However, this is costly and has a risk of thrombotic events. We have generated 'Supercharged' *in-vitro* derived platelets, containing rFVIIa. These were created using a lentivirus vector, which presents a barrier for clinical use.

We sought to create an inducible cell line (driven by the Von willebrand factor (vW) signal peptide domain 2 (SPD2), fused to the gene of interest), driven by an *integrin aIIb* promoter) which expresses rFVIIa, without the use of a lentiviral vector, which has the potential for clinical use.

Methods

Intracellular flow cytometry and a thrombin generation assay were used to determine whether lentiviral transduced *in-vitro* derived platelets contain rFVIIa.

Using cloning and lipofectamine transfection (using a triple plasmid system), we have inserted a HS-insulator containing construct with vWF-SPD2-VEGF into ROSA26 of one of our inducible iPSC cell lines.

Results

We have shown, using flow cytometry, that *in-vitro* generated platelets virally transduced with rFVIIa express rFVIIa and increase thrombin production, compared with platelets from untransduced MKs.

In preliminary data, we have shown that MKs generated with the triple plasmid system express VEGF, as a proof of principle.

Conclusion

We have demonstrated that *in-vitro* generated platelets 'supercharged' with rFVIIa contain rFVIIa and have a functional effect. We are currently transfecting iPSC's with the triple plasmid system to generate inducible supercharged platelets, which express rFVIIa.

P20

Interference of ticagrelor with thromboxane B_2 measurement by enzyme-linked immunosorbent assay

Katie O'Connor[1], Heather M Judge[2], Nimisha Shaji[2], Georgia M Williamson[2], Robert F Storey[2], William A E Parker[2]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Sheffield, Sheffield, United Kingdom

Abstract

Introduction

Measurement of serum thromboxane B_2 (TXB_2) is the gold-standard method for assessing the inhibition of platelet cyclooxygenase-1 by aspirin. Commercial enzyme-linked immunosorbent assay (ELISA) kits for TXB_2 measurement are available. In patients with high-risk coronary syndromes, aspirin may be prescribed alongside ticagrelor, a $P2Y_{12}$ inhibitor. Peak serum levels of ticagrelor with standard dosing are around 1µmol/L. Based on an initial observation, we investigated whether ticagrelor interferes with TXB_2 measurement by two commonly-used ELISA kits.

Methods

TXB_2 ELISAs from RnD Systems and Cayman Chemical were evaluated using standard TXB_2 solutions and a 96-well plate reader (ThermoSkan). Standard TXB_2 detection curves were generated with added ticagrelor (1µmol/L) or vehicle. A ticagrelor dose-response curve (0.003-1µmol/L) was also constructed. The assay was run with TXB_2 (0.084 ng/mL) and ticagrelor, cangrelor, or vehicle added to serum from aspirin-treated blood. Ticagrelor or vehicle was introduced at different steps throughout the ELISA protocol to identify at which point interference occurred.

Results

Ticagrelor had no effect on TXB_2 detection by the Cayman kit, but completely abolished detection by the RnD kit. There was a clear dose-response effect, but even 0.01µmol/L-ticagrelor partially interfered with TXB_2 measurement. Addition to serum of ticagrelor, but not cangrelor or vehicle, similarly blocked TXB_2 detection. Stepwise addition of ticagrelor during the assay revealed that interference occurred at any stage before but not after the plate washing step.

Conclusions

When measuring TXB_2 in the presence of ticagrelor, use of the RnD kit should be avoided due to severe interference with the assay.

P21

The effect of hyperglycaemia on platelet responses *in vitro*.

Mr Jordan-Lee Greaves[1], Dr Giordano Pula[1], Professor Thozhukat Sathyapalan[2], Professor Tim Palmer[1], Dr Ahmed Aburima[1]

[1]Biomedical Institute for Multimorbidity, Hull York Medical School, Hull, United Kingdom. [2]Clinical Sciences Centre, Hull York Medical School, Hull, United Kingdom

Abstract

Cardiovascular complications are twice as likely to cause fatalities in patients with Type 2-Diabetes Mellitus (T2DM) compared to the rest of the population. An emerging role for platelet hyperactivity has been hypothesised, although the underlying molecular mechanisms remain to be elucidated. To assess whether poor glycaemic control is sufficient to increase platelet responsiveness, we tested the effect of the manipulation of glucose concentration on platelet activity *in vitro*. Washed platelets or platelet-rich plasma (PRP) were incubated for up to 24 hours with different concentrations of glucose: 2.5mM (hypoglycaemia), 5mM (normoglycaemia), 10mM (hyperglycaemia) and 20mM (severe hyperglycaemia). The osmolarity of the washed platelet suspension was adjusted with mannitol. Platelet responsiveness was tested by light transmission aggregometry (LTA) using thrombin, U46619 (thromboxane A2 analogue), collagen, PAR1 agonist TRAP-6 or adenosine diphosphate (ADP) as agonists. Static platelet adhesion in response to fibrinogen and collagen was also tested. Surprisingly, hyperglycaemic conditions did not significantly affect platelet aggregation in washed platelets. However, the aggregation of platelets in response to collagen (3µg/mL) or ADP (1µM) was potentiated in PRP. These results suggest that experiments on washed platelets are unlikely to be a representative *in vitro* model for platelet hyperactivity in T2DM. Interestingly, hyperglycaemic conditions significantly increased the adhesion of platelets to fibrinogen and collagen in static conditions, although platelet surface area and circularity were unaffected. Taken together, our data suggest that hyperglycaemia has the potential to increase platelet responsiveness *in vitro* and that the choice of the correct experimental conditions is essential to study this phenomenon.

P22

Cryopreservation of fluorescently labelled platelets for remote platelet function analysis.

Maria V Diaz Alonso, Neline Kriek, Joanne L Dunster, Tanya Sage, Chris I Jones, Jonathan M Gibbins

University of Reading, Reading, United Kingdom

Abstract

Platelet Phenomic Analysis (PPA) is a flow cytometry-based platform that enables multi-parameter analysis of platelet function, including sensitivity to activators (EC50) and response capacity (maximal minus minimal response). However, its use in multi-centre studies, is limited due to the need of specialised equipment at site of patient recruitment. We aimed to address this by developing an approach for cryopreservation of stimulated and antibody-labelled platelets.

To achieve this, bespoke reagents and protocols were developed for long-term frozen storage prior to centralised analysis, facilitating platelet function profiling for clinical applications and drug trials.

Platelets were activated with various concentrations of ADP, CRP or TRAP-6, in the presence of labelled anti-fibrinogen and anti-P-selectin antibodies in microtiter plates. By optimising for cell morphology, antibody labelling, and fluorophore stability, we developed a reagent for treating samples before freezing at -80°C. Samples were analysed by flow cytometry immediately or frozen for up to 3 months before defrosting, cell washing, and analysis. PPA analysis software was used to extract the sensitivity and capacity parameters.

Stimulation of platelets led to a concentration-dependent increase in P-selectin exposure and fibrinogen binding. Sensitivity and capacity data pre- and post-freezing (up to 3 months) were found to be nearly indistinguishable. Correlation analysis at 3 months (ADP, CRP, TRAP-6; sensitivity, capacity) revealed $R>0.95$, $p<0.01$.

The new capability to freeze samples for later centralised flow cytometry analysis will enable powerful PPA multi-parameter analysis for platelet function to be incorporated into multi-centre studies or used in locations lacking flow cytometry expertise.

P23

Establishing a microfluidic model of thrombus and endothelial cell interaction under flow as a tool for application of novel imaging techniques

Jocelyn M Auger[1], Emma L Faulkner[1], Claudia Schönichen[2], Judith MEM Cosemans[2], Steve G Thomas[1], Natalie S Poulter[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Maastricht University, Maastricht, Netherlands

Abstract

The integration of endothelial cells (ECs) into *in vitro* flow chamber assays has recently been established by various research groups to more closely replicate physiological conditions within a blood vessel. Thrombi do not form when blood flows over an intact EC layer; however, thrombi develop upon stimulation of ECs (simulating inflammation) or disruption of the EC layer (simulating injury or atherosclerotic plaque rupture). The incorporation of ECs into the model has improved physiological relevance; nevertheless, obtaining detailed imaging of platelets within a three-dimensional densely packed thrombus, as well as observing alterations in EC morphology, remains challenging.

To address similar challenges, we have applied a new technique called expansion microscopy (ExM) to thrombi within flow chambers. ExM facilitates nanoscale imaging of cells and tissues through the physical enlargement of samples. The fourfold isotropic expansion, combined with optical clearing of the sample, permits enhanced imaging volume and depth. ExM samples can be imaged via a variety of microscopy techniques to extract different types of information, including cell size and the distribution of intracellular proteins. We have recently demonstrated the ability to visualise sub-cellular structures and quantitatively characterise platelets within thrombi.

We now aim to combine ExM with other super-resolution microscopy techniques to understand the impact of incorporating ECs into flow chambers on thrombus formation. We will analyse detailed platelet and EC morphology, such as size, shape, activation status and localisation of cytoskeleton or cell membrane receptors, and investigate how these are altered when ECs are damaged or activated.

P24

Platelet hyperactivity in Diabetes Mellitus patients: a potential role of platelet-derived extracellular vesicles.

Christopher Sennett[1], Prathy Sasikumar[2], Jon Gibbins[2], Giordano Pula[1]

[1]Hull York Medical School, Hull, United Kingdom. [2]University of Reading, Reading, United Kingdom

Abstract

Platelet dysfunction is a key factor in the development of cardiovascular comorbidities of diabetes. Interestingly diabetes patients have also shown to respond less favorably to antiplatelet drugs. The molecular mechanisms increasing the thrombotic risk and reducing the efficacy of antiplatelet in diabetes patients remain to be elucidated. Platelet-derived extracellular vesicles (pEVs) are increased in the blood of diabetes patients and may contribute to the thrombotic risk associated with this disease.

We utilized quantitative bottom-up proteomics to assess the proteome of platelets and pEVs in diabetes patients and compared it to age- and sex-matched healthy donors. We identified 114 differentially expressed proteins in platelets, and 48 differentially expressed proteins in pEVs. Upregulated proteins in platelets are involved in platelet degranulation, innate immune system interaction and the immune response, intracellular signaling and platelet hyperactivity. Surprisingly, some established proteins regulating membrane trafficking and mitochondrial function and some key receptors such as GPIX and GPIbB were downregulated. In pEVs, we observed a significant upregulation of proteins potentially involved with platelet activation and inflammation, such as pro-platelet basic protein (PPBP) and Junctional adhesion molecule A (JAM-1).

Overall, the analysis of platelet and pEV proteomes highlights potential mechanisms responsible for platelet hyperactivity and increased thrombosis risk in diabetes. Characterising the mechanisms increasing the risk of thrombosis in diabetes will drive the development or repurposing of drugs to fight the cardiovascular co-morbidities of this disease. This will increase the life expectancy of patients with diabetes and reduce the financial burden of this disease on the health system.

P25

Investigating the effects of platelet microparticle derived cargo in chronic kidney disease responses.

James Salt, Wayne Roberts

Leeds Beckett University, Leeds, United Kingdom

Abstract

Platelet microparticles (PMPs) are small vesicles shed from activated or apoptotic platelets, accounting for the majority of circulating MPs. In Chronic Kidney Disease (CKD), elevated production of PMPs increases the risk of cardiovascular complications and directly drives CKD progression by driving renal inflammation and fibrosis. The underpinning mechanisms are yet to be elucidated; however it is likely delivery of PMP bioactive cargo to renal cells having an important role. The goal of this research is to systematically evaluate the effects of PMP derived content on renal proximal tubular epithelial cells responses associated with CKD.

PMPs were isolated from thrombin stimulated platelets and their providence verified using nanoparticle tracking and flow cytometric analysis. Using immuno-fluorescence microscopy we have shown that PMPs are internalised by primary human renal proximal tubular epithelial cells (hRPTECs). hRPTECs were chosen as they are a major part of the kidney, susceptible to hypoxia, proteinuria and fibrosis, that are considered driving forces in the progression of kidney diseases. Following incubation with PMPs for 72 hours, hRPTECs proliferation was significantly reduced, with fewer cells in the S phase of the cell cycle. hRPTECs treated with PMPs for 24 hours exhibited significant global changes in gene expression; genes implicated with cell proliferation and DNA synthesis were down regulated whilst genes relating to fibrosis, extracellular matrix production and inflammation were significantly elevated. Therefore, the data reinforces the hypothesis that PMP derived bioactive cargo contributes to CKD progression, by way of decreasing cell proliferation and increasing fibrosis, key hallmarks of CKD pathogenesis.

P26

Platelet-derived serotonin drives lung immunopathology in aged SARS-CoV-2 infected mice

Davide Marotta[1], Chiara Perucchini[2], Marta Grillo[1], Leonardo Giustini[2], Chiara Malpighi[2], Valeria Fumagalli[1], Micol Ravà[2], Violette Mouro[1], Lorenza Donnici[3], Raffaele De Francesco[3], Luca Guidotti[2], Matteo Iannacone[1,2], Marco De Giovanni[2,1]

[1]Vita-Salute San Raffaele University, Milan, Italy. [2]San Raffaele Scientific Institute, Milan, Italy. [3]INGM - Istituto Nazionale di Genetica Molecolare, Milan, Italy

Abstract

Severe COVID-19 remains a major threat to elderly and immunocompromised individuals, who are also at risk for long-term complications. Despite available antiviral therapies, severe disease outcomes persist, emphasizing the need to better understand its underlying mechanisms.

To address this, we developed an age-dependent severe COVID-19 mouse model by infecting young (7–9 weeks) and aged (9+ months) C57BL/6 mice with a mouse-adapted SARS-CoV-2 strain. Severe disease, characterized by weight loss and impaired lung function, was observed exclusively in aged mice, which exhibited an imbalanced immune response and hyperactivated platelets. Notably, anti-platelet therapy and platelet depletion significantly improved disease outcomes in aged mice, highlighting a detrimental role of platelets in severe COVID-19.

We then investigated serotonin, a platelet-derived mediator of activation and aggregation. Aged mice released significantly higher serotonin levels upon stimulation compared to young adults. Depleting platelet serotonin using fluoxetine, a selective serotonin reuptake inhibitor, completely reversed severe disease symptoms in aged mice, restoring body weight and lung function to levels comparable to young mice. Notably, fluoxetine treatment reduced platelet activation but did not alter innate immunity, demonstrating a platelet-specific effect.

Ultimately, hyperactivated platelets in aged mice contributed to increased fibrin deposition, leading to lung dysfunction. These findings identify platelet-serotonin interactions as a key driver of severe COVID-19 and suggest targeting this pathway as a potential therapeutic strategy. In line with these results, we are currently evaluating platelet-serotonin receptor inhibitors in aged mice as a novel approach to mitigate severe COVID-19 pathology.

P27

Semi-automated analysis of single platelet calcium signalling dynamics using CalPeaks

Abigail Grainger, Joanne. L Dunster, Kirk. A. Taylor

University of Reading, Reading, United Kingdom

Abstract

Background: Calcium is a critical second messenger regulating the rate of platelet activation, and ion channels may serve as antithrombotic targets. Conventional calcium signalling studies rely upon average measurements from cellular suspensions. Though increasing throughput, this approach does not enable interrogation of single-cell calcium dynamics and direct inter-cellular calcium signalling. Single-cell measurements are limited by signal-noise ratio and data processing times.

Aim: To develop a semi-automated analysis package to quantify single-cell calcium peaks and synchronising behaviour.

Methods: Fluo4-stained platelets were spread onto fibrinogen and visualised by confocal microscopy. Platelets were activated by application of TRAP6 (10μM) plus $CaCl_2$ (1mM). Calcium signals were exported and analysed via in-house developed software (calPeaks). This software identifies calcium spikes from background noise, extracts spike magnitude, width, and amplitude, and calculates synchronicity between cells.

Results: TRAP6-evoked calcium responses were recorded in platelets pre-treated with secondary signalling blockers (acetylsalicylic acid, apyrase, cangrelor, NF449 & MRS2179), gap junction inhibitor (carbenoxolone) or vehicle control. Regions of interest (ROI) were drawn around the platelet perimeter, and fluorescence values were exported. Cells were scored as ‘paired’ or ‘unpaired’, and data were processed using CalPeaks. Similar numbers of peaks were recorded under each condition, but carbenoxolone reduced peak synchronicity and magnitude (P=0.0064), whilst secondary blockers reduced the mean rise time (P=0.0021).

Conclusion: CalPeaks is a bespoke, user-friendly analysis package for rapidly evaluating single-cell calcium spike dynamics and inter-cellular communication. This approach reduces analytical bias and standardises evaluation of key calcium spike metrics. Future applications include characterising antiplatelet therapies.

P28

Dietary intake of zinc in humans regulates platelet stores and modulates haemostatic function

Um-May Sumya[1], Claire S Whyte[1], John Beattie[2], Frank Thies[2], Nicola J Mutch[1]

[1]The Insitute of Medical Sciences, School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Aberdeen, United Kingdom. [2]The Rowett Institute, School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Aberdeen, United Kingdom

Abstract

BACKGROUND

Platelets harbour the main circulating pool of free zinc that is secreted following stimulation. Zinc is known to be a cofactor for several haemostatic proteins and can influence platelet reactivity.

AIM

To investigate if dietary restriction of zinc modulates platelet levels and haemostasis.

METHODS

Thirty-six healthy volunteers on a habitual diet were recruited. Dietary intake of zinc was restricted for 2 weeks (1 mg/day) followed by repletion (≥4.5 mg/day) for 2 weeks. Blood was collected at each phase and platelet rich plasma (PRP) isolated for haemostatic assays. Levels of zinc in washed platelet lysates were quantified by inductively coupled mass spectrometry.

RESULTS

Zinc levels were lower in platelets in the depletion compared to the habitual phase (6.78 ± 0.52 vs. 9.63 ± 1.19 µM, $p<0.05$). Collagen-induced platelet aggregation was attenuated by zinc depletion (45.06 ± 6.9 vs 90.80 ± 6.62 %, $p<0.0001$) and lag time to thrombin generation was longer (16.39 ± 4.05 vs. 13.07 ± 1.07 min, $p<0.05$). The rate of clot growth (37.52 ± 3.02 vs 51.55 ± 2.35 µm/min, $p<0.01$) and clot retraction were attenuated by zinc depletion ($p<0.001$). Lysis of PRP-rich clots was slower upon zinc depletion (99.46 ± 5.19 vs. 78.30 ± 3.85 min, $p<0.001$). Repletion of zinc into the diet of volunteers returned all haemostatic parameters and platelet levels to baseline.

CONCLUSION

This is the first evidence in humans that the platelet stores of zinc are directly modulated by dietary intake. In turn, the levels of zinc in platelets regulate the haemostatic response, potentially explaining bleeding complications in zinc deficient individuals

P29

Investigation of the mechanisms and significance of degranulation-dependent Zn2+ release following platelet activation

Emily Capes[1], Havovi Chichger[1], Joanna-Marie Dear[2], Matthew Harper[2], Nicholas Pugh[1]

[1]Anglia Ruskin University, Cambridge, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

Following vascular injury or pathogenic thrombosis formation, platelet activation results in the release of bioactive agents including zinc (Zn^{2+}) that acts as a platelet agonist. Zn^{2+} has been found to accumulate in atherosclerotic plaques to levels six-fold greater than surrounding tissues, potentially providing a reservoir accessible to haemostatic processes following plaque rupture. Sub-activatory concentrations (30μM) of Zn^{2+} potentiate platelets to activation by conventional agonists. These concentrations are not considerably greater than basal plasma Zn^{2+} (0.5-1μM) and could feasibly be attained as a result of platelet degranulation, cell damage, or atherosclerotic plaque rupture. The aim of this work was to investigate the mechanisms involved in of Zn^{2+} induced platelet aggregation, and to quantify the total Zn^{2+} released from platelets following activation. Experiments were performed to investigate of the effects of Zn^{2+} on buffer components in calcium-free Tyrodes buffer. Platelet activation was measured in washed human platelet suspensions using light transmission aggregometry and flow cytometry. Zinc release was quantified using optical emission spectroscopy and colorimetric assay. It was found that Zn^{2+} induced platelet aggregation was aberrated in the absence of phosphate in the buffer whilst all other normal platelet responses were maintained. Optical emission spectroscopy quantified Zn^{2+} release from platelets following activation. Further investigations will quantify total Zn^{2+} released from platelets after stimulation from various agonists.

P30

Characterisation of Platelet Sensitivity in Immune Thrombocytopenia Identifies Distinct Subgroups with either Higher or Lower Platelet Sensitivity

Rocel Saputil[1], Joanne Dunster[2], Neline Kriek[2], Michelle MH Tan[1], Alicia Au[1], Amna Malik[1], Alice Hart[1,3], Cynthia Lee[3], Spencer Mackie[3], Tung Le[3], Wayne A Mitchell[1], Jon Gibbins*[2], Nichola Cooper*[1,3]

[1]Imperial College London, London, United Kingdom. [2]University of Reading, Reading, United Kingdom. [3]Imperial College Healthcare NHS Trust, London, United Kingdom

Abstract

Background: Immune Thrombocytopenia (ITP) is an autoimmune disorder characterised by a low platelet count (≤100×10^9/L). In approximately 60% of patients, anti-platelet autoantibodies target glycoproteins Ib-IX and IIb/IIIa, leading to platelet destruction and impaired production. Current treatments, such as thrombopoietin receptor agonists (TPO-RAs), aim to increase platelet count. More recently, SYK and BTK inhibitors have been introduced. However, ITP diagnosis and treatment remain a challenge due to its heterogeneity. While some patients experience minimal symptoms, others have significant bleeding risks, and paradoxically, some may develop thrombosis. This highlights the need for personalised therapeutic approaches.

The Platelet Phenomic Analysis (PPAnalysis) assesses platelet function using flow cytometry. We hypothesised that this assay could categorise patients at higher risk of bleeding or thrombosis.

Methods: Patients and controls were recruited from the ITP Centre at Imperial College Healthcare NHS Trust. Whole blood samples were processed using PPAnalysis. In brief, platelet-rich plasma (PRP) was labelled with PE-Cy5-conjugated P-Selectin as marker of α-granule secretion, and stimulated with increasing concentrations of platelet agonists, adenosine diphosphate (ADP; 0.03-30μM), collagen-related peptide (CRP; 0.003-3μg/mL), and thrombin receptor activator peptide 6 (TRAP-6; 0.05-15μM). Platelet sensitivities, measured as LogEC50, were analysed using R.

Results and Conclusions: ITP patients display either higher or lower platelet sensitivity compared to controls. This variability was independent of platelet count, sex, or treatment, suggesting distinct subgroups. This emphasises the role of aberrant platelet reactivity in ITP pathogenesis, and suggests PPAnalysis as a valuable tool for classifying patients and guiding personalised treatment strategies in ITP.

P31

Platelet activation in anti-PF4 antibody mediated conditions associated with thrombosis and thrombocytopenia

Jovita Manickam, Richard Buka, Phillip Nicolson, Samantha Montague

University of Birmingham, Birmingham, United Kingdom

Abstract

Background:

Heparin-induced thrombocytopenia (HIT) and vaccine-induced immune thrombocytopenia and thrombosis (VITT) are examples of thrombotic thrombocytopenia syndromes (TTS), that are mediated by immune-complex driven platelet activation. In these conditions, anti-platelet factor 4 (anti-PF4) antibodies form immune complexes with PF4 (and heparin in HIT), that activate platelets via FcγRIIA. Other patients have TTS of different aetiologies that are associated with thrombosis and thrombocytopenia, which are also potentially anti-PF4 antibody driven.

Aims:

To investigate whether serum from non-HIT/VITT TTS patients activate platelets and explore whether this involves FcγRIIA and anti-PF4 antibodies.

Methods:

We tested whether serum samples from 5 TTS and 2 suspected VITT/HIT patients activated healthy donor platelets by light transmission aggregometry and flow cytometry. Washed platelets ($2x10^8$/mL) were exposed to patient serum with and without heparin/PF4 and ± IV.3 (10 µg/mL; FcγRIIA blocker) (n=3-6).

Results:

TTS2 and TTS4 serum (disseminated intravascular coagulation) with and without PF4 mediated aggregation and activation in >50% of donors tested and showed reduction with IV.3 in some donors. TTS1 (haemolytic uraemic syndrome) only induced aggregation/activation in one responsive donor. TTS3 (pancreatitis-associated thrombotic microangiopathy) and TTS5 (unknown diagnosis) did not activate platelets. HIT/VITT serum (with heparin/PF4) mediated aggregation and activation in all donors tested and was blocked with IV.3.

Conclusion:

VITT and HIT serum mediated robust aggregation/activation. TTS serum gave diverse activation responses across samples and donors, potentially related to the clinical condition. Additional testing for serum anti-PF4 levels is required to determine roles for anti-PF4 antibodies.

P32

Development of platelet aggregation and calcium flux assays in a microplate format to determine agonist and antagonist activities.

Simon Lydford[1], Andrew Johnson[2], Wendy Tomlinson[2], Natalia Dovlatova[2], Andrea Townsend-Nicholson[3]

[1]Molecular Devices UK Ltd, Wokingham, United Kingdom. [2]Platelet Services Ltd, Nottingham, United Kingdom. [3]University College London, London, United Kingdom

Abstract

Platelets are small, anucleate blood cells essential for haemostasis, aggregating at injury sites to form thrombi. They also play roles in immune responses and tumour regulation. However, dysregulated platelet activation can lead to thrombotic diseases such as heart attacks and ischaemic stroke. Thrombus formation involves three stages: adhesion (platelets bind to exposed matrix proteins), activation (shape change and chemical secretion), and aggregation (platelet-to-platelet adhesion). Platelet activation is triggered by collagen binding to glycoprotein VI (GPVI) and subsequent stimulation by agonists such as ADP and TxA2, which activate G-protein-coupled receptors (GPCRs). This leads to phospholipase C (PLC)-mediated calcium (Ca^{2+}) release, crucial for cytoskeletal reorganisation, shape change and integrin degranulation.

Understanding how ligands modulate platelet function in healthy and diseased patients will help improve the understanding of their role in blood clotting and other conditions, guiding the development of safe and efficacious therapies.

Here, we demonstrate a high-throughput microplate-based approach for measuring Ca^{2+} flux and platelet aggregation using ADP, CRP, and U46619. Assays were performed on the FlexStation 3, iD3 Multi-Mode Microplate Readers, and the FLIPR High-Throughput Cellular Screening System. Miniaturising from cuvette-based platelet suspensions to 96- and 384-well microplates enabled rapid and reliable determination of EC_{50} and IC_{50} values while reducing platelet and reagent consumption. This approach enhances experimental efficiency allowing for the testing of multiple conditions with smaller sample volumes, opens up the possibility of small animal species platelet studies and aligns with NC3Rs principles by reducing animal use in research.

P33

Lipopolysaccharide potentiates platelet activation through Proline Rich Tyrosine Kinase Pyk2 engagement

Michela Gemme[1], Serena Rustichelli[2,1], Valentina Bonaldi[1], Giosia Rossignod[1], Marta Zarà[1], Gianni Francesco Guidetti[1], Mauro Torti[1], Ilaria Canobbio[1]

[1]Department of Biology and Biotechnology - University of Pavia, Pavia, Italy. [2]University school for Advanced Studies IUSS, Pavia, Italy

Abstract

The ability of lipopolysaccharide (LPS) to promote platelet activation and aggregation has been widely investigated in the past with controversial results (Galgano et al, 2022). Some studies demonstrate that LPS enhances platelet activation induced by classical agonists in vitro (Lopes et al., 2017) via TLR4 engagement and PI3K and ERK signaling. The proline rich tyrosine kinase Pyk2 is known to regulate PI3K and MAPK in platelets downstream of integrin, but its involvement in LPS-induced platelet activation has never been tested.

In this study, we have investigated the effect of LPS on platelet activation and the role of Pyk2 in this process.

Washed platelets from WT and Pyk2 KO mice were preincubated with LPS (10 µg/mL) for 10 minutes and stimulated with TRAP4 (0.5 mM) for 5 minutes. Platelet aggregation and activation of signaling proteins were investigated in light aggregometry and immunoblotting respectively.

We have demonstrated that LPS potentiates, but does not directly activate, platelets to low doses agonist. Preincubation with LPS enhances both aggregation and the phosphorylation of Akt, GSK3 and ERK1/2, but not p38MAPK. LPS also increases the phosphorylation of Pyk2. Interestingly, the potentiation of aggregation and activation induced by LPS is lost in the absence of Pyk2.

Our study demonstrates that Pyk2 is involved in the priming effect of LPS on TRAP4-induced platelet aggregation and activation.

P34

Investigation on the role of phosphoinositide 3-kinases in the biogenesis of platelet-derived extracellular vesicles

Valentina Bonaldi[1], Marta Zarà[1], Giosia Rossignod[1], Serena Rustichelli[1,2], Michela Gemme[1], Ilaria Canobbio[1], Sonia Severin[3], Mauro Torti[1], Gianni Francesco Guidetti[1]

[1]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy. [2]University School for Advanced Studies IUSS Pavia, Pavia, Italy. [3]Inserm, U1048 and University of Toulouse, I2MC, Toulouse, France

Abstract

Platelet extracellular vesicles (PEVs) are heterogenous, membrane-surrounded particles involved in several biological processes, including hemostasis and thrombosis (Melki *et al*., 2017). Despite their importance, little is known about the molecular mechanisms regulating PEV biogenesis and release from platelets. The different isoforms of phosphoinositide 3-kinases (PI3Ks) play a key role in regulating platelet functional responses, including membrane dynamics and vesicular trafficking, which are potentially relevant for PEV biogenesis (Jackson *et al*., 2004). In this study we aim at investigating the role of PI3Ks in PEV biogenesis.

Human platelets were stimulated with different soluble agonists (thrombin, U46619 and convulxin) either in the absence or in the presence of the pan-PI3K inhibitor wortmannin, or isoform-specific inhibitors. PEVs released under the different conditions were isolated by ultracentrifugation and analyzed by BCA protein assay, Nanoparticle Tracking Analysis (NTA) and western blotting.

PI3K inhibition was first assessed in whole platelets by evaluating AKT phosphorylation and platelet aggregation, confirming the predominant roles of PI3Kβ and PI3Kγ. Protein quantification and nanoparticle tracking analysis (NTA) revealed that thrombin- and convulxin-induced PEV production was affected by wortmannin. Complete PI3K inhibition with wortmannin also led to moderate changes in PEV size and their composition of key signal transduction enzymes.

In conclusion, PI3Ks appear to play important and unknown roles in PEV biogenesis, release, and cargo selection. Ongoing studies aim to define the impact of PI3Ks on molecular composition and functions of PEVs.

P35

Generation and testing of Novel PROTAC Tools For Targeting Protein Kinase Degradation in Human Platelets

Hetty Walker[1], Justin Trory[1], Jordan Vautrinot[1], Dean Wheeler[2], Graham Marsh[2], Joel Cresser-Brown[2], Hannah Maple[2], Varinder Aggarwal[1], Ingeborg Hers[1]

[1]University of Bristol, Bristol, United Kingdom. [2]Tocris Bio-techne, Bristol, United Kingdom

Abstract

Platelets are anucleated so current methods for identifying therapeutic treatment strategies are challenging, time consuming and may require the use of animal studies. PROTACs are heterobifunctional molecules which work by hijacking the endogenous ubiquitination proteasomal system to degrade specific proteins, including those that contribute to disease like thrombosis. Due to a platelet's lack of nucleus, targeted protein degradation can create a human platelet 'knockout', as degraded proteins cannot be regenerated.

Novel PROTAC compounds have been optimised, synthesised and tested based on computational studies. The main protein targets are the closely related tyrosine kinases Focal Adesion Kinase (FAK) and PYK2, which are believed to contribute to specific signalling mechanisms within platelets.

Synthesised PROTACs have been tested for their ability to degrade their target protein using western blotting analysis. Treatment of platelets with these novel PROTACs resulted in significant FAK degradation compared to untreated controls, giving over 85% degradation efficacy and high specificity at nanomolar concentrations.

A PROTAC degrading FAK and PYK2 with a degradation efficacy of over 90% has also been evaluated. Platelet a-granule secretion and integrin $a_{IIb}b_3$ activation was not affected upon agonist stimulation in FAK and PYK2-deficient platelets. In contrast, platelet spreading on fibrinogen coated surfaces showed that the later stages of lamellipodia formation are significantly impaired in FAK and PYK2 deficient platelets.

These results provide evidence that PROTAC compounds can be optimised to target protein degradation in platelets and used as an investigative tool to study specific signalling pathways and their role in platelet function.

P36

Preliminary results from a platelet reactivity investigation in Parkinson's disease reveal trends of novel differences in both nitric oxide sensitivity and aggregation responses to several agonists

Marcela Usmari Moraes[1], Eloise Marriott[2], Valia Rodriguez[1], Sarah Harris[3], Richard Buka[4], John-Peter Ganda Mall[5], Sharon Muzerengi[3], Ilse Pienaar[2], Caroline Kardeby[1]

[1]Aston University, Birmingham, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom. [3]University Hospital Birmingham Foundation Trust, Birmingham, United Kingdom. [4]Sandwell and West Birmingham Hospitals NHS Trust, Birmingham, United Kingdom. [5]Örebro University, Örebro, Sweden

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative condition affecting over 8.9 million people worldwide by 2021, according to estimates by the World Health Organisation. It is characterised by loss of dopaminergic innervation in the midbrain's *Substantia Nigra pars compacta*, with intracellular aggregation of the synaptic-regulating protein, α-synuclein, playing a contributory role. Recent evidence highlights a >30% prevalence of deep-vein thrombosis and a ~3-fold increase in the risk of ischaemic stroke in PD patients. However, mechanisms underlying such comorbid vascular events occurring during PD remain to be determined. Our study hypothesised that the PD-related protein, α-synuclein, may contribute to platelet reactivity, thereby damaging the endothelial cell layer of microvessels to render PD patients more susceptible to vascular pathology. To investigate this further, we are recruiting individuals living with PD and healthy volunteers to measure platelet activation and aggregation levels using flow cytometry and lumi-aggregometry, respectively. Our preliminary results (n = 6 on submission date) show an increased level of platelet aggregation to low concentrations of several platelet agonists, with a trend in correlation between years since diagnosis and decreased nitric oxide sensitivity. The exact mechanism for such increased activation may be attributed to higher levels of circulating α-synuclein in PD patients. Our investigation into a novel pathological synergism between increased α-synuclein levels and changed human platelet responses is ongoing. The preliminary findings show promise to greatly increase understanding as to how platelets could act as a pathophysiological contributor in proteinopathies and serve as a target for novel therapeutic interventions

P37

In vitro generation and *in vivo* validation of functional VEGFA-supercharged platelets for promoting cardiac repair post-myocardial infarction.

Samantha Mason[1], Annett Mueller[1], Solomon Shepherd[1], Aramis Khorchidian[1], Alexi Crosby[1], Holly Foster[1], Winnie Lau[1], Lily Du[2], David Wilcox[2], Alastair Poole[2], Cedric Ghevaert[1]

[1]Cambridge Stem Cell Institute, Cambridge, United Kingdom. [2]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, USA

Abstract

Background

Ischaemic heart disease is a leading cause of death globally. Although early reperfusion has greatly improved pa9ent outcomes following myocardial infarc9on (MI), post-MI cardiac remodelling leads to eventual heart failure. The pro-angiogenic factor VEGFA has shown promise in myocardial regenera9on, although disappoin9ng clinical efficacy may stem from inadequate delivery. Here, we present supercharged-platelets (Super-PLTs), a delivery system for VEGFA cardiac therapy.

Methods

Recombinant VEGFA was assembled into an expression casseMe under the control of a CD41 promoter (for early megakaryopoie9c-specific expression) and fused to the SD2 domain of VWF, promo9ng a-granule localisa9on. Super-PLTs were produced via len9viral transduc9on of forward programmed megakaryocytes (FoP-MK) and genomic integra9on of recombinant DNA construct into the ROSA26 locus of the FoP-MK iPSC line. Super-PLT payloads were detected, quan9fied, and func9onally validated.

Results

We demonstrate that len9virally-transduced MKs were capable of proplatelet forma9on. We detected VEGFA expression in Super-PLTs by flow cytometry and immunostaining and quan9fied a pharmacologically-ac9ve 9ter by ELISA. We then confirm VEGFA a-granule localisa9on by immunostaining. We show Super-PLTs are ac9va9on and degranula9on competent and that they significantly improve percentage closure in a scratch-wound assay of a HUVEC culture. Finally, we present significant therapeu9c efficacy of Super-PLTs in a murine ischaemia-reperfusion model.

Conclusion

We present methods for supercharging iPSC-derived platelets with the pro-angiogenic factor VEGFA, confirm func9onality in vitro, and propose Super-PLTs as an experimental therapy for post-MI myocardial regenera9on. We an9cipate that Super-PLT technology will be generalisable to other biologics and applicable to a range of indica9ons

P38

Selective TLR4 antagonists (FP7 and FP12) modulate thromboinflammatory responses

Jomanah Alqallaf[1], Sakthivel Vaiyapuri[1], Ketan Patel[1], Francesco Peri[2]

[1]University of Reading, Reading, United Kingdom. [2]University of Milan, Milan, Italy

Abstract

Platelets are essential not only in haemostasis and thrombosis but also in the induction of inflammatory responses through reacting to various inflammatory molecules in different pathological conditions. Current antiplatelet medications demonstrate efficacy in treating cardiovascular diseases, particularly thrombotic conditions. Nonetheless, they are linked to a considerable risk of bleeding complications. Thus, a deeper understanding of the role of platelets in developing thromboinflammatory conditions will facilitate the discovery of safe antiplatelet medications. Lipopolysaccharide (LPS) molecules interact with toll-like receptor 4 (TLR4) on platelets and various immune cells and trigger inflammatory responses. Consequently, TLR4 serves as a potential therapeutic target for managing thromboinflammatory conditions. This study aims to investigate the effects of LPS-derived Francisco Peri compounds (FPs), FP7 and FP12, that are known to inhibit TLR4 signalling on the modulation of platelet function. We examined the impact of FPs on platelet activation using human platelet-rich plasma and isolated platelets by light transmission aggregometry and flow cytometry by measuring the levels of fibrinogen binding and P-selectin exposure. Both FP molecules inhibited CRP-XL and ADP-induced platelet aggregation in a dose-dependent manner. Similarly, they reduced the levels of fibrinogen binding and P-selectin exposure upon activation with an agonist. We demonstrated that FP molecules mitigate agonist-induced platelet activation through binding to TLR4. Due to their synthetic nature and precise actions on TLR4, these molecules may have the potential to become novel antiplatelet agents to control thrombotic as well as platelet-mediated inflammatory responses under diverse pathological settings.

P39

Investigating how differential methylation of ZFHX3 is associated with increased risk of cardiovascular disease.

Chloe Stephenson-Deakin[1], Natalia Cullell[2], Jurek Krupinski[1,2], Israel Fernandez[2], Tristan McKay[1], Amanda Unsworth[3], Steve White[4]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Fundació Docència i Recerca MútuaTerrassa, Barcelona, Spain. [3]University of Leeds, Leeds, United Kingdom. [4]University of Newcastle, Newcastle, United Kingdom

Abstract

Cardiovascular diseases account for high levels of global morbidity and mortality. Multiple Genome and Epigenome-Wide Association Studies (GWAS and EWAS) have identified that the regulation of ZFHX3, a transcription factor, is significantly associated with a range of cardiovascular diseases associated with thrombosis, including cardioembolic stroke, atrial fibrillation, and myocardial infarction. Two transcripts of ZFHX3 are expressed in humans, ZFHX3-201 and ZFHX3-203, however, the functional implications of expression of the different transcripts in the mechanisms that control thrombosis is unknown. Hypomethylation of the EWAS site, cg07786668, is significantly associated with an increased risk of ischemic stroke and is located proximally in the first intron of the ZFHX3-203 transcript. This project aims to define the impact of methylation of this EWAS site on the expression of ZFHX3-203 and its function in megakaryocytes and platelets using a cell-line based approach. MEG01 cells treated with 5-Azacytidine, an inhibitor of global DNA methylation, show a significant increase in expression of total ZFHX3 and ZFHX3-203 and has no effect on viability. Overexpression of ZFHX3-203 in MEG01 cells has been achieved and RNA-sequencing will be performed to interrogate changes in regulators of megakaryocyte biology and platelet function and identify mechanisms by which ZFHX3 increases cardiovascular risk.

P40

LifeAct-encapsulated fusogenic liposomes: an innovative approach to enable dynamic imaging of the human platelet actin cytoskeleton

Yan Wu[1], Carly Kempster[2], Joshua Cull[1], Ying Yang[3], Alan Harper[3], Alice Pollitt[1]

[1]University of Reading, Reading, United Kingdom. [2]Xap Therapeutics Ltd, Cambridge, United Kingdom. [3]Keele University, Keele, United Kingdom

Abstract

Introduction: Platelets play an important role in many physiological and pathological processes. Understanding the molecular mechanisms that underlie platelet function is important for the identification of improved therapeutics. However, platelets lack a nucleus which means that conventional methods, used to study molecular processes, cannot be directly used. Fusogenic liposomes offer a mechanism to deliver compounds, including imaging probes, directly into the cytoplasm of human platelets.

Aims: This project aims to deliver LifeAct conjugated to a 488 nm fluorescent label (Lifeact-488) into human platelets by fusogenic liposome mediated delivery. LifeAct-488 is an imaging probe that enables the visualisation of actin cytoskeletal dynamics.

Methods: The morphology of fusogenic liposomes was assessed using FIB-SEM. The impact of liposome fusion on platelet function was determined by assessing P-selectin exposure using flow cytometry. Confocal microscopy was used to capture live-images during liposome fusion and the delivery of LifeAct-488 into human platelets.

Results: The fusion of liposomes with platelets does not lead to platelet activation and normal platelet functions are preserved. LifeAct-488 delivery into human platelets using liposomes does not result in platelet activation as measured by P-selectin exposure. Normal P-selectin exposure following CRP-XL mediated platelet activation is maintained. Fusogenic liposomes can efficiently deliver LifeAct-488 into human platelets enabling live-cell imaging of the actin cytoskeleton.

Conclusions: These results demonstrate that LifeAct-encapsulated fusogenic liposomes can be used as an effective method to visualise the actin cytoskeleton of human platelets. This approach has the potential to deliver other compounds directly into the cytoplasm of platelets.

P41

Standardisation and optimisation of in vitro thrombus formation image analysis using ThrombusMetrics.

Tyler L Horn, Harry Simmonds, Valentina Shpakova, Maria V Diaz Alonso, Abigail Grainger, Jonathan M Gibbins, Craig E Hughes, Kirk A Taylor

University of Reading, Reading, United Kingdom

Abstract

Background: *In vitro* thrombus formation using microfluidic chips is routinely used to study platelet function. This enables analysis of shear force on thrombi formed from healthy volunteers and patients with cardiovascular disease. However, the approaches taken to generate thrombi and the subsequent analyses performed lack standardisation. Standardising these approaches would improve comparisons between datasets from different laboratories.

Aim: To develop an analysis pipeline to standardise in vitro thrombus formation data outputs.

Approach: We automated the extraction of key metrics using open-source (FIJI, Image J) or proprietary (Nikon NIS-Elements) software. Outputs were processed using an open-source analysis pipeline (ThrombusMetrics; Python). Our script uses the Pandas library to analyse macro-derived CSV files, the script cleans raw data and calculates the key parameters into a tabular format.

Results: Macros were developed in FIJI to extract thrombus surface coverage (% area & mm2), volume (mm3), height (µm) and real-time adhesion (f/f0). A separate automated pipeline was designed to extract these parameters using AI-driven analysis features in NIS elements. Batch analysis was incorporated to streamline high-throughput datasets. NIS elements processing was faster than FIJI (7.7s/image vs 15.4s/image). ThrombusMetrics pipeline was validated by comparing control and cangrelor-treated samples.

Conclusion: We present a pipeline to streamline high-throughput analysis of thrombus formation data. Nikon processing was two-times faster than FIJI but relies upon proprietary software. Therefore, FIJI is preferred for standardisation across the field. This approach enables rapid and robust analysis of thrombus formation datasets with applications for basic and clinical research.

P42

Investigating arginine methylation as a novel post-translational modification of the platelet integrin, αIIbβ3

Angel Garcia, Francisco Rivero, Pedro Beltran-Alvarez

Centre for Biomedicine, Hull York Medical School, University of Hull, Hull, United Kingdom

Abstract

Clinical trials involving PRMT inhibitors have reported bleeding-related side effects, suggesting that arginine methylation (ArgMe) may play a critical regulatory role in platelet function. Supporting this hypothesis, our previous work demonstrated that PRMT1 inhibitors reduce the expression and activation of key platelet receptors, including αIIb. However, the molecular mechanisms underlying the role of ArgMe in platelet biology and thrombosis remain poorly understood.

To investigate the regulatory role of ArgMe in platelet function, we first sought to identify conserved ArgMe sites in aIIbβ3. Using mass spectrometry, we analysed αIIb and β3 isolated from unstimulated, collagen-stimulated (10µg/ml), and thrombin-stimulated (0.1U/ml) platelets. This revealed >4,500 ArgMe sites across >700 proteins, with 41 sites identified in αIIbβ3. Among these, 15 sites in aIIb and 15 in β3 were conserved across all 10 donors regardless of stimulation state. Importantly, genetic variations in three of these sites, αIIb-R551, β3-R63, and β3-R242, have been reported in patients with Glanzmann thrombasthenia, a bleeding disorder characterised by a deficiency in functional αIIbβ3. These findings suggest that ArgMe of aIIb**β**3 may be essential for normal platelet activity.

This study highlights the potential significance of ArgMe in platelet biology and provides a foundation for exploring its role in bleeding disorders. Ongoing research focuses on evaluating the functional relevance of these sites through functional assays of cells transfected with plasmids encoding αIIbβ3 bearing arginine-to-lysine mutations at the identified ArgMe sites. These efforts aim to elucidate the mechanistic link between ArgMe and platelet dysfunction, offering potential therapeutic insights for conditions like Glanzmann thrombasthenia.

P43

Immunophenotypic analysis of age-defined platelet sub-populations in mice

Ami Vadgama, Nicola Dark, Harriet Allan, Timothy Warner, Paul Armstrong

Queen Mary University of London, London, United Kingdom

Abstract

Background: Circulating platelet sub-populations are currently of interest. Newly formed platelets are more haemostatically active, and flow cytometry-based immunophenotyping is used to characterise surface receptors that confer function.

Aim: Couple multi-parametric immunophenotyping cytometry with *in vivo* temporal labelling of platelets to interrogate differential receptor expression and functional responses of age-defined platelet sub-populations.

Methods: Fluorescent anti-CD42c antibodies were injected (i.v) into C57BL/6 mice at various timepoints to define platelet sub-populations by age. Platelet rich plasma from diluted and centrifuged blood, was incubated with PAR-4 amide (AYGKPF), thromboxane A_2 mimetic (U46619), or vehicle in the presence of 12 fluorescently labelled antibodies. Spectral flow cytometry (Cytek Aurora) simultaneously determined 14 physical and receptor parameters per platelet (100,000 per condition). Data were analysed using NovoExpress, Omiq and Graphpad Prism.

Results: Following platelet activation, surface marker expression varied according to agonist stimulation. Generally, up to 11 parameters (CD9, CD29, CD31, CD36, CD41, CD49b, CD62P, CD63, CD107a, CD29, CD41, JON-A) increased, while up to 3 markers (FSC, SSC, GPVI) decreased. Newly formed platelets (<24 hours) expressed more CD62P, and CD107a in response to activation compared to older circulating platelets (>24 hours), which expressed more CD63. Under both basal and activation conditions, newly formed platelets had differential expression of 6 parameters (FSC, CD31, CD9, CD49b, GPVI, H2).

Conclusion: We present a novel approach that can characterise age-defined platelets across 14 parameters without the use of RNA stains, in low-volume samples. This approach will be powerful for detecting, tracking and studying platelet subpopulations within *in vivo* disease models.

P44

Development of zafirlukast analogues for improved anti-thrombotic activity through thiol isomerase inhibition

Sabeeya Khan[1], Justine Keovilay[2], Kaitlind Howard[3], Kirk Taylor[1], Sienna Wurl[2], Melanie Szahaj[2], Tanya Sage[1], Nishad Chandrika[3], Caxia Hou[3], Oleg Tsodikov[3], Sylvie Garneau-Tsodikova[3], Daniel Kennedy[2], Jonathan Gibbins[1]

[1]University of Reading, Reading, United Kingdom. [2]Western New England University, Springfield, USA. [3]University of Kentucky, Lexington, USA

Abstract

Background:

Thiol isomerases play essential and non-redundant roles in platelet activation, aggregation, and thrombus formation. Thiol isomerase inhibitors have the potential to overcome two major drawbacks of current antithrombotic therapies, as they target both arterial and venous thrombosis without enhancing bleeding risks. Recently, an FDA-approved drug, zafirlukast (ZAF), was shown to be a promising pan-thiol isomerase inhibitor. The objective of this study is to develop analogues of ZAF with optimized thiol isomerase inhibition and antithrombotic activity.

Methods:

35 ZAF analogues were tested in an insulin turbidometric assay for thiol isomerase inhibition. Analogues were tested for platelet activation, aggregation, P-selectin expression, and laser-induced thrombosis in mice and compared with the parent compound.

Results:

Of the 35 analogues, 12 retained activity. Compound 21 demonstrated a greater potency, 5 had a similar potency, and 6 had a weaker potency than that of ZAF. Analogues demonstrated inhibition of platelet aggregation and P-selectin expression as compared to ZAF, consistent with their potencies. ZAF and compound 21 were shown to be reversible inhibitors of thiol isomerases, and not cytotoxic to cultured, lung, liver, and kidney cells. Finally, in an in vivo assessment of thrombus formation, compound 21 was able to significantly inhibit thrombus formation without affecting bleeding times.

Conclusions:

ZAF analogue, compound 21, with properties superior to those of ZAF, was synthesized, demonstrating improved inhibition of platelet activation, aggregation, and thrombus formation as compared to the parent ZAF. This approach could yield a promising clinical candidate for treatment and prophylaxis of arterial and venous thrombosis.

P45

Nitric oxide regulates platelet microparticle formation

Alex Hodgkinson, Matt Hindle, Wayne Roberts

Leeds Beckett university, Leeds, United Kingdom

Abstract

Platelet Microparticles (PMPs) are the most abundant microparticle circulating in the blood. The level of PMPs increase in a range of inflammatory disorders including atherosclerosis, uncontrolled diabetes and cancer. Increased PMP levels correlate with the severity of these diseases and altered PMP cargo in these conditions directly contributes to disease progression. Despite this, the mechanisms regulating PMP formation remain relatively unexplored. In this project we investigate the capacity of the endogenous platelet inhibitor nitric oxide (NO) to regulate PMP formation.

Our data show that both platelet adhesion and aggregation, key steps underpinning thrombus formation, result in PMP formation. Stimulation with typical platelet agonists further enhanced PMP formation and PMP size, with thrombin inducing the greatest PMP release. Crucially, NO donor GSNO was able to reduce PMP formation and in addition to this, NO reduced the size of PMPs produced by agonist treatment. Critically, this inhibition was reversed by the soluble guanylyl cyclase inhibitor ODQ. These data suggest that NO, through cGMP dependent signalling, reduces PMP formation, changes the phenotype characteristics and alters resultant PMP cargo.

This work characterises for the first time the regulation of PMP formation and function by NO, which could have implications for pathologies driven, in part, by aberrant PMP formation.

P46

The novel PGI2 analogue ligand 9 has superb ability to inhibit platelet function and treat severe pulmonary arterial hypertension.

Megan F. Allen[1], Shahida Mallah[1], Changcheng Jing[2], Carl May[1], Charles E. Laurent[3], Stuart J. Mundell[1], Varinder K. Aggarwal[2], Ingeborg Hers[1]

[1]University of Bristol, School of PPN, Bristol, United Kingdom. [2]University of Bristol, School of Chemistry, Bristol, United Kingdom. [3]IPS Therapeutique Incorporated, Sherbrooke, Canada

Abstract

Prostacyclin is a potent platelet inhibitor and vasodilator acting on the prostacyclin IP receptor. PGI_2 and their analogues are widely prescribed as anti-platelet drugs and in the treatment of pulmonary arterial hypertension (PAH), however their lack of stability and selectivity limits their clinical application.

In this study, we therefore synthesised a range of PGI_2 analogues with excellent chemical stability and tested their ability to inhibit platelet function and treat pulmonary arterial hypertension. PGI_2 analogues demonstrated superb potency (pM-nM range) in inducing VASP phosphorylation and inhibiting platelet aggregation, integrin activation, P-selectin expression and *in vitro* thrombosis, with the effect blocked by the IP receptor antagonist CAY10441. Using wire myography, our lead compound ligand 9 demonstrated higher potency in initiating vasodilation of pulmonary arteries from Sprague-Dawley rats than the clinically used IP receptor agonists iloprost and ralinipag. Furthermore, ligand 9 dose dependently reversed the reduction in pulmonary arterial and right ventricular pressure, cardiac output and velocity time interval in the Sprague-Dawley Sugen hypoxia PAH model, to the level of normoxic controls, whereas systemic blood pressure and heart rate were unaffected.

In conclusion, we identified the highly potent and efficacious PGI_2 analogue Ligand 9, which showed superb ability to inhibit platelet function, vasodilate pulmonary arteries and reverse PAH in a Sugen hypoxia PAH model. These findings highlight Ligand 9 as a promising therapeutic candidate for the treatment of thrombosis and pulmonary arterial hypertension.

P47

Platelet reactivity is associated with visual memory performance in healthy older adults

Mishaal Rehman[1], Gabriella Rossetti[1], Eva Feredoes[2], Eleanor Benford[3], Joanne Dunster[2], Jonathon Gibbins[2], Anastasia Christakou[2]

[1]Manchester metropolitan university, Manchester, United Kingdom. [2]University of Reading, Reading, United Kingdom. [3]University of Manchester, Manchester, United Kingdom

Abstract

Platelet function is associated with cardiovascular and neurocognitive decline in ageing. Our previous research linked platelet reactivity to neurovascular function in older adults. Here, we examine its relationship with cognitive function and demographics.

Fifty-two healthy adults (50–80 years, BMI = 25.4 (4.1), 28 female) participated. We quantified platelet reactivity using a plate-based aggregation assay and assessed verbal memory via the Rey Auditory Verbal Learning Task (RAVLT) and visual short-term memory using the Oxford Memory Test (OMT, n=26).

BMI and age were unrelated to platelet reactivity. However, females exhibited significantly higher platelet reactivity than males (Cohen's d = 0.938, p = 0.010). More men fell into the low-reactivity group, while more women were in the high-reactivity group ($\chi^2 = 6.58$, p = 0.037). Differences appeared in platelet aggregation responses to agonists but persisted across all agonist-inhibitor combinations, with females showing higher platelet capacity ($p < 0.05$).

Platelet reactivity did not correlate with verbal memory, but higher reactivity was linked to poorer visual memory. Increased absolute error on the OMT correlated with higher overall platelet reactivity (R = 0.55, p = 0.009) and sensitivity to adenosine 5'-diphosphate (R = 0.75, $p < 0.001$). No demographic variables were associated with cognitive function, but controlling for them slightly strengthened the platelet-visual memory relationship.

In conclusion, elevated platelet reactivity is linked to poorer visual short-term memory in healthy older adults. While sex differences were observed in platelet reactivity, demographics (age, sex, and BMI) did not influence cognitive performance.

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Characterising the composition and thrombogenicity of human coronary artery endothelial cell (HCAEC) derived extracellular matrix (ECM) under healthy and disease conditions.

Amelia Drysdale[1], Jon Humphries[1], Amanda Unsworth[2], Stephen White[3], Sarah Jones[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]University of Leeds, Leeds, United Kingdom. [3]Newcastle University, Newcastle, United Kingdom

Abstract

Background: Atherosclerotic plaque rupture or erosion leads to exposure of ECM proteins, and subsequent platelet adhesion and thrombus formation. To identify novel antithrombotic targets, studies routinely use Type I HORM collagen *in-vitro* or expose the ECM in young healthy murine vessels *in-vivo*. The aims of this study were 1) to develop an *in-vitro* thrombosis model that incorporates human, disease-relevant ECM and 2) to characterise the composition and thrombogenicity of HCAEC-derived ECM under healthy and disease associated conditions.

Methods: HCAECs were cultured for 7 days, with 3 days of treatment (TNF-α or 10% cigarette smoke extract). Decellularisation was performed, leaving an intact matrix for platelet adhesion, spreading and thrombus formation assays. The HCAEC ECM was then characterised using mass spectrometry. Proteomic analysis was performed using Proteome Discoverer, DAVID bioinformatics tool and Graphpad Prism.

Results: Thrombi generated on native ECM were smaller but more abundant compared with Type I HORM collagen ($P<0.01$), consistent with results from platelet adhesion assays. TNF-α treatment resulted in the formation of larger thrombi ($P<0.05$) and increased platelet adhesion ($P<0.05$) on the ECM compared to control. ECM composition varied significantly between healthy and dysfunctional cells, with a reduction in structural collagens observed ($P<0.001$), associated with platelet GPVI, and significant increases in coagulation proteins, including tissue factor, plasminogen and Factor XIII ($P<0.0001$).

Conclusions: Endothelial dysfunction significantly alters the composition of ECM produced by HCAECs, increasing thrombogenicity. Investigating thrombus formation using human disease relevant ECM may accelerate the discovery of novel antithrombotic drugs which disrupt thrombosis but not haemostasis.