EUROTHROMBOSIS SCIENCE 2008

December 11, 12, 13
Barcelona, Spain

Thrombosis
ESC Working Group

EUROPEAN SOCIETY OF CARDIOLOGY®

ORAL COMMUNICATIONS

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December 11th - Oral Communications – Session I

17:00 - 17:15
THE THROMBOXANE RECEPTOR PATHWAY IS INVOLVED IN TISSUE FACTOR EXPRESSION AND NADP(H) OXIDASE ACTIVATION IN STIMULATED ENDOTHELIAL CELLS
S. Del Turco*, G. Basta*, G. Lazzerini*, L. Chancharme§, L. Lerond§, R. De Caterina*#
*CNR Institute of Clinical Physiology, Pisa, #Chair of Cardiology, G. d'Annunzio University, Chieti, Italy, and §Institut de Recherches Internationales Servier

17:15 – 17:30
ISCHAEMIC AND THROMBOTIC EFFECTS OF AIR POLLUTION
N.L Mills, A.J Lucking, N.A Boon, T. Sandstrom, A. Blomberg, K. A. Fox, D. E. Newby
Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, UK, Department of Respiratory Medicine and Allergy, Umeå University, Umeå, Sweden

17:30 – 17:45
PLATELET RESPONSE TO ASPIRIN IS REDUCED IN PATIENTS WITH PREVIOUS MYOCARDIAL INFARCTION COMPARED TO PATIENTS WITH CORONARY ARTERY DISEASE
S. B. Larsen[1], S. B. Mortensen[1], E. L. Grove[1], AM Hvas[2], S. D. Kristensen[1].
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17:45 – 18:00
PATHOPHYSIOLOGICAL CORRELATES OF LOW HAEMOGLOBIN CONCENTRATIONS IN PATIENTS WITH ISCHEMIC HEART DISEASE
G. Coluzzi, F. Marzo, A. Lavorgna, S. Cecchetti, E. Santucci, T. Rio, F. Andreotti.
Institute of Cardiology, Catholic University Medical School, Rome, Italy.

18:00 – 18:15
G148A–A NON CONSERVATIVE POLYMORPHISM OF THE GLYCOPROTEIN 130 IS ASSOCIATED WITH CORONARY ARTERY DISEASE
K. Thaler[1], A. Wonnert[1], P. Hohensinner[1], S. Boresch[2], K. Katsaros[1], C. Kaun[1], K. Huber[3], J Wojta[1], G Maurer[1], **TW Weiss**[3].
[1] Department of Internal Medicine II; Medical University of Vienna, Austria, [2] Department of Computational Biological Chemistry, [3] Department for Cardiology and Emergency Medicine, Wilhelminenspital, Vienna, Austria

18:15 – 18:30
A RANDOMIZED DOUBLE-BLIND STUDY ON THE EFFECTS OF ATORVASTATIN AND ROSUVASTATIN ON OXIDATIVE STRESS AND INFLAMMATION AS DETERMINANTS OF *IN VIVO* PLATELET ACTIVATION IN PATIENTS WITH HYPERCHOLESTEROLEMIA: RELEVANCE OF 3'UTR/T LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 POLYMORPHISM
N. Vazzana*, L. Puccetti‡, F. Santilli*, F. Bruni‡, AL. Pasqui‡, St. Lattanzio*, L. Pietrangelo*, F. Ciani°, G. Ciabattoni*, A. Auteri‡, G. Davì*.
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THE THROMBOXANE RECEPTOR PATHWAY IS INVOLVED IN TISSUE FACTOR EXPRESSION AND NADP(H) OXIDASE ACTIVATION IN STIMULATED ENDOTHELIAL CELLS

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Background: Tissue factor (TF) expression and surface exposure are key events in thrombosis, likely contributing to clinical events in vascular disease. Thromboxane (TX)A_2, an unstable metabolite of arachidonic acid released from multiple cell types, is known for its pro-aggregating and vasoconstrictor properties. It exerts its effects via TP (TX- prostaglandin endoperoxide) receptor, also expressed in endothelial cells. The TP receptor antagonist S 18886 demonstrated antithrombotic effects, but also inhibited the increased adhesion molecule expression and the increased nitric oxide synthase expression in activated endothelial cells. As the underlying molecular mechanisms are largely unexplored, we studied the effects of TP agonism and of antagonism on TF expression and procoagulant activity in human umbilical vein endothelial cells (HUVEC), and intracellular signal transduction pathways involved.

Methods and Results: HUVEC ± 30 min pretreatment with the TP antagonist S 18886 were stimulated with the TP receptor agonist U 46619 or TNF-α for 6 hours. TF total expression and surface exposure were assessed by enzyme immunoassays, and TF-dependent procoagulant activity by the generation of Factor Xa. HUVEC exposed to U 46619 featured a concentration-dependent increase in TF total expression and surface exposure. These were associated with enhanced procoagulant activity. S 18886 (1 µmol/L) significantly reduced U 46619 (1 µM)-induced TF expression (-20% ± 7%, $P<0.05$) and procoagulant activity (-32% ± 11%, $P<0.05$). More interestingly, S 18886 (1 µmol/L) prevented the increase of TF expression after TNF-α (20 ng/mL) stimulation (-25% ± 9%, $P<0.05$). Both U 46619- and TNF-α-induced TF expression were mediated by the increase of intracellular reactive oxygen species (ROS), assessed by a dichlorofluoresceine-based assay, and this was inhibited by S 18886 (-44% ± 6% and -24% ± 5% $P<0.05$, respectively). S 18886 decreased NADP(H) oxidase activity and the membrane association of its p47-phox component, accounting for the reduced production of ROS.

Conclusions: Our results demonstrate that endothelial TP receptor mediate TF expression, surface exposure and activity mediated both by TP agonists and by a widely acting stimulus, such as TNF-α, thus implicating endogenous TP agonists in mediating TNF-α endothelial activation. This occurs through NADP(H) oxidase activation and the consequent generation of ROS.

ISCHAEMIC AND THROMBOTIC EFFECTS OF AIR POLLUTION

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Purpose: : Although the mechanisms are unknown, it has been suggested that transient exposure to traffic derived air pollution may be a trigger for acute myocardial infarction. We conducted controlled exposures to dilute diesel exhaust in healthy volunteers and patients with stable coronary heart disease to determine the direct effect of air pollution on vascular function, thrombus formation and myocardial ischaemia in man.

Methods: Using a double-blind randomized crossover design, 20 male healthy volunteers and 20 men with prior myocardial infarction were exposed to dilute diesel exhaust (300µg/m3) or filtered air during periods of rest and moderate exercise in a controlled exposure facility. In healthy volunteers, thrombus formation was measured at two and six hours following exposure using the Badimon ex vivo perfusion chamber. In patients, myocardial ischemia was quantified by ST-segment analysis using continuous 12-lead electrocardiography during the exposure, and vascular vasomotor and fibrinolytic function was assessed using intra-arterial agonist infusions six hours after the exposure.

Results: Compared to filtered air, diesel exhaust inhalation increased thrombus formation under low and high shear conditions by 24.2% (95% confidence interval [CI], 13.5% to 35.0%, P<0.001) and 19.1% (95% CI, 10.5% to 27.8%, P<0.001) respectively at two and six hours. Exposure to diesel exhaust did not aggravate pre-existing vasomotor dysfunction in patients with coronary heart disease, but did reduce acute endothelial tissue plasminogen activator release (P<0.05; area under the curve decreased by 35%). Exercise induced ST-segment depression was present in all patients but there was a greater increase in ischemic burden during exposure to diesel exhaust (-22±4 versus -8±6mVs, P<0.001).

Conclusions: Inhalation of diesel exhaust inhibits endogenous fibrinolytic capacity and increases ex vivo thrombus formation in man. Furthermore, diesel exhaust promotes exercise induced myocardial ischemia in men with stable coronary heart disease. Our findings have identified ischemic and thrombotic mechanisms for the observations that exposure to combustion-derived air pollution is associated with adverse cardiovascular events including acute myocardial infarction.

Key words: air pollution, fibrinolysis, thrombosis

EUROTHROMBOSIS SCIENCE 2008: FOCUS ATHEROTHROMBOSIS
December 11, 12, 13 – Barcelona (Spain)

PLATELET RESPONSE TO ASPIRIN IS REDUCED IN PATIENTS WITH PREVIOUS MYOCARDIAL INFARCTION COMPARED TO PATIENTS WITH CORONARY ARTERY DISEASE

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Introduction: Aspirin reduces the risk of thrombotic events in patients with coronary artery disease (CAD). However, several studies report a variable platelet response to aspirin.

Aim: To investigate whether the platelet response to aspirin was different in a) healthy subjects, b) patients with CAD but no previous myocardial infarction (MI), c) patients with one previous MI and d) patients with more than one previous MI despite aspirin therapy.

Methods: We included 33 healthy subjects and 174 patients: n_{CAD}=60; $n_{\text{patients with one previous MI}}$=59; $n_{\text{patients with more than one previous MI}}$=55. Patients with previous MI were all treated with aspirin *before* the event. Prior to blood sampling, all participants received 75 mg of non-enteric coated aspirin daily for 7 days. Compliance was optimized by pill counting and interviews. Aspirin responsiveness was assessed as Area Under the Curve (AUC) by the Multiplate® whole blood platelet aggregation analyzer (Dynabyte, Germany). Platelet aggregation was induced by arachidonic acid 1.0 mM and by collagen 1.0 µg/mL. Furthermore, P-selectin was measured with ELISA (R&D Systems, USA)

Results: AUC for arachidonic acid indicated by median AUC values (25%;75%) was: healthy subjects: 81 (68;111); CAD: 97 (63;169); one MI: 165 (97;272); more than one MI: 157 (95;201), p=0.0017 (ANOVA). We found no difference in AUC between patients with one MI and more than one MI (p=0.45), whereas platelet response to aspirin was significantly lower in patients with previous MI (n=114) compared with healthy subjects (p=0.0015) and CAD patients (p=0.0023). The same pattern was found for collagen: healthy subjects: 188 (123;310); CAD: 225 (171;356); one MI: 309 (215;428); more than one MI: 276 (198;426), p=0.0070 (ANOVA). As for arachidonic acid, no difference in AUC between patients with one MI and more than one MI was seen (p=0.38). Platelet response to aspirin was significantly lower in patients with previous MI compared with healthy subjects (p=0.0019) and CAD patients (p=0.0301). P-selectin values (median ng/ml, [25%;75%]): healthy subjects: 54 (46;68); CAD: 67 (56;85); one MI: 71 (58;93); more than one MI: 73 (57;87), p=0.0033 (ANOVA). P-selectin was significantly increased in patients compared with healthy subjects, p=0.0003.

Conclusion: Platelet response to aspirin assessed by Multiplate® was lower in patients with previous MI compared with healthy subjects, and P-selectin was increased in all patients compared with healthy subjects indicating an increased platelet activation in patients. Patients with previous MI had a lower platelet response to aspirin when compared to CAD patients without previous MI.

Keywords: Aspirin, Platelets, Myocardial Infarction

PATHOPHYSIOLOGICAL CORRELATES OF LOW HAEMOGLOBIN CONCENTRATIONS IN PATIENTS WITH ISCHEMIC HEART DISEASE

G. Coluzzi, F. Marzo, A. Lavorgna, S. Cecchetti, E. Santucci, T. Rio, F. Andreotti.
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Purpose. Low haemoglobin concentrations ([Hb]), kidney dysfunction and reduced circulating progenitor cells (PCs) are consistently emerging as independent predictors of adverse outcomes in patients with ischemic heart disease (IHD). Reduced [Hb] may reflect a general depression of hematopoiesis. Nitric oxide (NO), being involved in erythropoietin's biological activity and in PC mobilisation, contributes to regulate [Hb]. We investigated the relation among [Hb], NO bioavailability, circulating PCs and kidney dysfunction in IHD patients.

Methods. The study was conducted in 2 phases: 1) an exploration population of 83 consecutive patients (57 with acute coronary syndrome, 26 with chronic stable angina) grouped *a priori* into those with [Hb] on admission <13 g/dl (n=17) and those with [Hb]≥13 g/dl (n=66) in whom NO metabolites (NOx, uM) were measured ≥48 hours after admission; 2) a validation population of 54 similar consecutive patients (41 with acute coronary syndrome, 13 with chronic stable angina) in whom CD34+ circulating levels and glomerular filtration rate (ml/min/1.73 m^2) were additionally evaluated . Exclusion criteria for both populations were age>80 years, left ventricular ejection fraction (LVEF)<30%, liver, lung or renal failure, active bleeding, iron deficiency, abnormal red cell [Hb] or volume.

Results. In the exploration population, patients with [Hb]<13g/dl versus those ≥13 g/dl did not differ significantly in age, risk factors, serum creatinine, LVEF, number of coronary vessel disease, clinical diagnosis and medications (all Ps ≥0.17). In contrast NOx values were 15.1±2.6 vs 24.3±2.1 uM (P=0.009). On multivariate regression, lower NOx remained an independent predictor of low [Hb] (P=0.01 in all patients, P=0.04 in each gender). Conversely, C-reactive protein (P=0.33), white cell count on admission (P=0.35), age (P=0.89), clinical diagnosis, left ventricular hypertrophy (P=0.28), and treatment with aspirin (P=0.70) did not predict [Hb]. In the validation population, routine clinical and biochemical variables (including [Hb] on admission) did not differ significantly between diagnostic groups. CD34+ average levels were 10.7±2.9 cells/ul in patients with acute coronary syndrome vs 6.8±2.2 cells/ul in patients with chronic stable angina (P=0.67). CD34+ were lower among the 22 patients with anemia vs the 32 non-anemic patients (4.0±0.8 vs 14.0±3.7 cells/ul, P=0.044). Admission values of [Hb] were associated with GFR (P=0.002, r=0.39). Finally, [Hb] (P=0.009, beta=0.42), but not GFR (P=0.65), significantly predicted number of circulating CD34+.

Conclusion. Taken together, these data document an association between lower [Hb], impaired NO bioavailability and reduced circulating CD34+ among patients with IHD. Low [Hb], both in the presence and absence of kidney disease, may predict adverse outcomes in IHD patients, through reduced NO bioavailability and reduced circulating levels of PCs.

Key words: Haemoglobin, nitric oxide, circulating progenitor cells

G148A–A NON CONSERVATIVE POLYMORPHISM OF THE GLYCOPROTEIN 130 IS ASSOCIATED WITH CORONARY ARTERY DISEASE

K. Thaler[1], A. Wonnert[1], P. Hohensinner[1], S. Boresch[2], K. Katsaros[1], C. Kaun[1], K. Huber[3], J Wojta[1], G Maurer[1], **TW Weiss[3]**.
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Purpose: The cytokines of the interleukin-6 (IL-6) family have been proven to play a pivotal role in inflammation, regulating the development and progression of atherosclerosis. They are characterized by their functional redundancy and pleiotropy, sharing a common signal transducing receptor subunit: glycoprotein 130 (gp130). The importance of individual variation in inflammatory response for atherosclerotic risk is becoming an increasingly interesting and exciting new frontier in cardiovascular research. Such variation is partially triggered by single nucleotide polymorphisms (SNPs). Therefore we investigated whether a non conservative SNP (G148A) of the gp130 gene affects the functional properties of the gp130 receptor and its possible association with coronary artery disease.

Methods: 522 patients, scheduled for elective coronary angiography were enrolled in this study. Absence (n=53) or presence (n=469) of coronary artery disease was assessed by coronary angiography. DNA was extracted from whole blood and gp130 polymorphism was detected by restriction fragment length analysis. We calculated structure refinement and solvent accessible surface of the gp130 using an *in silico* model.

Results: CAD was confirmed in 394 out of 445 (89%) carriers of the common G148G allele, in 70 out of 72 (97%) carriers of the heterozygous (G148A) and 5 out of 5 (100%) homozygous (A148A) carriers of the Arg allele. For hetero- and homozygous carriers of the Arg allele, univariate logistic regression revealed an Odds ratio of 4.85 (95% CI 1.15 -20.37, p=0.03) for coronary artery disease. This association remained significant after correction for age, sex, body mass index, diabetes, smoking, family history, hypertension, triglycerides, and total cholesterol levels in a multivariate logistic regression model. Using an *in silico* model, we could show that the G148A polymorphism induces a change in the solvent accessible surface of the gp130 receptor.

Conclusion: The role of the immune system in atherosclerosis is as complicated as the disease itself, and the majority of the complex immunological influences and interactions remain to be fully elucidated. Exactly this complexity, however, offers an explanation for the subtle, yet significant alteration in individual susceptibility to CAD caused by a protein alteration secondary to this SNP. The G148A polymorphism of gp130 correlates significantly with CAD. We speculate that this effect may derive from an alteration in the extracellular binding region of the receptor, resulting in a change in the affinity of the receptor for its ligands.

A RANDOMIZED DOUBLE-BLIND STUDY ON THE EFFECTS OF ATORVASTATIN AND ROSUVASTATIN ON OXIDATIVE STRESS AND INFLAMMATION AS DETERMINANTS OF *IN VIVO* PLATELET ACTIVATION IN PATIENTS WITH HYPERCHOLESTEROLEMIA: RELEVANCE OF 3'UTR/T LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 POLYMORPHISM

N. Vazzana*, L. Puccetti‡, F. Santilli*, F. Bruni‡, AL. Pasqui‡, St. Lattanzio*, L. Pietrangelo*, F. Ciani°, G. Ciabattoni*, A. Auteri‡, G. Davì*.
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Purpose. Concomitant with low-density-lipoprotein (LDL) cholesterol reduction, simvastatin causes a drastic reduction of *in vivo* platelet activation and oxidative stress in hypercholesterolemic patients.
We asked whether reduced platelet activation and oxidative stress and are related to a pleiotropic effect of any statin or to the LDL-lowering effect of treatment. Moreover, we evaluated whether a polymorphism (3'UTR/T) of lectin-like oxidized LDL receptor-1 (LOX-1) is associated with different antiplatelet effects during the two statin treatments.

Methods. We studied the effects of 2 structurally different statins in 60 hypercholesterolemic subjects, randomly assigned to a 8-week treatment with either atorvastatin 20 mg/die or rosuvastatin 10 mg/die. We measured at baseline, after 14 days and after 8-week treatment, plasma high sensitivity (hs)-CRP levels, and urinary excretion rate of 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2, *in vivo* markers of oxidative stress and platelet activation, respectively.

Results. After 8-week of treatment, both atorvastatin and rosuvastatin therapy were associated with comparable, significant ($p<0.0001$) reductions in LDL cholesterol (43.5%, p=ns), in plasma hs-CRP (from 0.89±10 to 0.81±0.1 mg/L, 9.3% vs. 0.88±0.1 to 0.77±0.1, 13.2%, p=ns) and urinary excretion rate of 11-dehydro-TXB_2 (1392±718 to 866±529, 37.5% vs. 1365±659 to 888±390 pg/mg creatinine, 30.5%, p=0.079), and 8-iso-$PGF_{2\alpha}$ (626±308 to 400±240 pg/mg creatinine, 29.4% vs. 610±249 to 436±169, 23.7%, p=0.15).
The % change in LDL cholesterol was significantly related to the % change in 11-dehydro-TXB_2 (Rho 0.34, $p<0.00001$), 8-iso-$PGF_{2\alpha}$ (Rho 0.36, $p<0.00001$) and CRP (Rho 0.38, $p<0.00001$).
No significant differences were observed in the presence of LOX-1 polymorphism.

Conclusion. We conclude that the effects of statins on platelet activation, oxidative stress and inflammation are unrelated to any intrinsic effect of either statin but are more likely mediated by their LDL-lowering action and not affected by the presence of LOX-1 polymorphism.

Keywords: hypercholesterolemia, statins, platelet activation.

ORAL COMMUNICATIONS

December 13th - Oral Communications – Session II

9:00 – 9:15
ROLE OF EPINEPHRINE IN THE ANTIPLATELET ACTION OF ASPIRIN AND P2Y12 RECEPTOR BLOCKADE IN PATIENTS WITH ACUTE CORONARY SYNDROME
A. Moscardo1, J. Valles1, M. Fuset2, M. Ruano2, M.T. Santos1,
1Research Center, 2UCI, Hospital La Fe, Valencia, Spain

9:15 – 9:30
THE NOVEL P-SELECTIN INHIBITOR PSI-697 REDUCES THROMBUS LOAD IN HEALTHY INDIVIDUALS IN AN *EX VIVO* MODEL OF THROMBOSIS
R. Chelliah, A. Lucking, K. Cortas, NJ. Beresford-Cleary, Newby D.
The University of Edimburgh

9:30–9:45
PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORγ INHIBITS ANGIOGENESIS BY SUPPRESSING CREB-MEDIATED CYCLOOXYGENASE-2 EXPRESSION IN HUMAN ENDOTHELIUM
E. Scoditti[1,2], M. Massaro[1,2], MA. Carluccio[1], A. Distante[1], C. Storelli[2], R. De Caterina[3].
[1]C.N.R. Institute of Clinical Physiology, Pisa and Lecce, Italy, [2]Department of Biology-University of Lecce, Italy, [3]Institute of Cardiology, "G. d'Annunzio" University, Chieti, Italy.

9:45 – 10:00
mCRP ENHANCES PLATELET DEPOSITION AND THROMBUS GROWTH UNDER FLOW; OPPOSING EFFECTS OF CRP ISOFORMS ON THROMBUS GROWTH
B. Molins[1,2], E. Peña[1], G. Vilahur[1], C. Mendieta[2], L. Badimon[1].
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10:00 – 10:15
DEVELOPMENT AND VALIDATION OF A RISK SCORE TO PREDICT SERIOUS BLEEDING IN STABLE OUTPATIENTS WITH ATHEROTHROMBOSIS
G. Ducrocq[1], J. Wallace[2], G. Baron[2], Ph. Ravaud[2], M. Alberts[3], P. Wilson[4], D. L. Bhatt[5], Philippe Gabriel Steg[1] on behalf of the REACH Registry Investigators.
[1] Departement de Cardiologie, Hôpital Bichat-Claude Bernard, Paris, France, [2] Departement d'Epidemiologie, Biostatistique et Recherche Clinique Hôpital Bichat-Claude Bernard, Paris, France, [3] Department of Neurology, Northwestern University Medical School, Chicago, USA, [4] Emory University, Atlanta, GA, [5] VA Boston Healthcare System and Brigham and Women's Hospital, Boston, MA, USA

10:15 – 10:30
THROMBIN UP REGULATES EXPRESSION OF ONCOSTATIN M (OSM) IN HUMAN MACROPHAGES AND PERIPHERAL BLOOD MONOCYTES
S.P. Kastl[1], W.S. Speidl[1], K.M. Katsaros[1], C. Kaun[1], K. Huber[2], J. Wojta[1].
[1] Department of Internal Medicine II, University of Vienna, [2] 3rd Department of Medicine, Wilhelminenspital, Vienna, Austria

11:30 – 11:45
INFLUENCE OF CALU A29809G POLYMORPHISM IN ATHEROMA PLAQUE CALCIFICATION: PROGNOSTIC IMPLICATIONS
D. Hernández-Romero, R. González-Conejero, JM. Ruíz-Nodar, A. Tello-Montoliu, M. Valdés, V. Vicente, V. Roldán, F. Marín.
Sº Cardiología. Hospital Universitario Virgen de la Arrixaca. Sº de Hematología y Oncología Médica. H.U. Morales Meseguer. Centro Regional de Hemodonación, Universidad de Murcia. Sº de Cardiología. Hospital General Universitario de Alicante. Spain

11:45 – 12:00
THE METABOLIC SYNDROME IS HIGHLY ASSOCIATED WITH INFLAMMATORY MARKERS AND ADIPOKINES IN ELDERLY MEN
M. Trøseid, I. Seljeflot, H. Arnesen,
Center for Clinical Heart Research, Department of Cardiology, Ullevål University Hospital, Oslo, Norway

12:00 – 12:15
SHORT-TERM MYOCARDIAL ISCHEMIA INDUCES CARDIAC mCRP EXPRESSION AND PRO-INFLAMMATORY GENE (Cox-2, MCP-1, and TF) UP-REGULATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS
G. Vilahur[*†], R. Hernández-Vera[*†], B. Molins[*†] ,L. Casaní [*†], X. Duran[*], T. Padró[*], L. Badimon[*†§].
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12:15 – 12:30
DOSE-EFFECT OF CLOPIDOGREL RE-LOADING IN PATIENTS ALREADY ON 75mg MAINTENANCE DOSE: THE RELOAD STUDY
J.P. Collet[1]; J. Silvain[1]; A. Landivier[1]; M.L. Tanguy[2]; G. Cayla[1]; A. Bellemain[1]; N. Vignolles[1]; S. Gallier[1]; F. Beygui[1]; **A. Pena**[1]; G. Montalescot[1].
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ROLE OF EPINEPHRINE IN THE ANTIPLATELET ACTION OF ASPIRIN AND P2Y12 RECEPTOR BLOCKADE IN PATIENTS WITH ACUTE CORONARY SYNDROME

A. Moscardo1, J. Valles1, M. Fuset2, M. Ruano2, M.T. Santos1,
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Purpose: Simultaneous signaling via several receptors adds complexity to the mechanisms of signal transduction in platelets. Receptor cooperation is specially important in G-protein coupled receptors (ADP, TXA2, epinephrine) and could amplify the effect of small concentrations of individual agonists. This could be of clinical importance in patients with acute coronary syndrome (ACS), where platelet activation and stress could be associated.

Aim: To evaluate in aspirin-treated ACS patients: a) effect of platelet co-stimulation with epinephrine (EPI) and arachidonic acid (AA) or EPI and ADP on the antiplatelet action of ASA; and b) to evaluate the role of PI3K in these dual receptor stimulation.

Methods and Patients: 27 ACS patients treated with aspirin within 48 hours of the acute event who had aggregation to AA blocked. Washed platelets were stimulated with EPI (10mM) + AA (200mM) or EPI+ADP (10mM). Aggregation was determined by optical aggregometry. TXA2 synthesis was evaluated by ELISA.

Results: Despite blockade of aggregation to AA, EPI + AA induced aggregation in 67% of patients (18/27). In these 18 patients, TXA2 synthesis was significantly increased by EPI (AA: 10.2±2.8 ng/ml; EPI+AA: 29.8±5.9 ng/ml; p=0.001), although TXA2 inhibition was still >95% vs. ASA-free normal subjects. This aggregation was abolished when TXA2 receptor was blocked with SQ29,248 (1mM). In addition, EPI reinforced ADP-induced aggregation and prevented its reduction when the P2Y12 receptor was blocked with 2MeSAMP (10mM). Importantly, PI3K inhibition with wortmannin (100 nM) simultaneously blocked EPI + AA induced aggregation and strongly reduced EPI + ADP-induced aggregation.

Conclusions: EPI reduced aspirin effectiveness in 67% of aspirin-treated ACS patients. This effect is associated with an increase in residual synthesis of TXA2, showing important functional effects. EPI also reduced the effect of P2Y12 blockade; this could be relevant for treatments that act in these receptors, like clopidogrel. Inhibition of PI3K corrected the prothrombotic effects of EPI and revealed that PI3K could be an important therapeutic target. (Grants FIS07/0463;RETICS-RD06/0026;MMA2006).

Key words: Acute coronary syndrome, aspirin, epinephrine

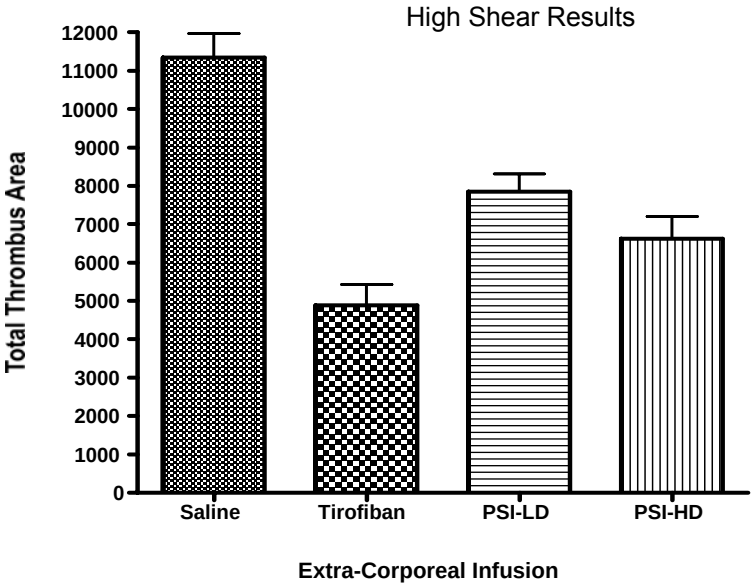
THE NOVEL P-SELECTIN INHIBITOR PSI-697 REDUCES THROMBUS LOAD IN HEALTHY INDIVIDUALS IN AN *EX VIVO* MODEL OF THROMBOSIS

R. Chelliah, A. Lucking, K. Cortas, NJ. Beresford-Cleary, Newby D.
The University of Edimburgh

Purpose: To investigate the ability of the novel P-Selectin inhibitor PSI-697 in reducing total thrombus area in healthy individuals under dynamic flow conditions, using an *ex vivo* model of thrombosis.

Method: Twelve healthy individuals were randomised to each receive PSI-697 at 20μM, PSI-697 at 2μM, Tirofiban 50ng/ml and saline as control. Using the Badimon *ex vivo* model of thrombosis, these agents were added to blood in the extra-corporeal circuit. There was a total of 5 minutes of perfusion time over the denuded porcine aorta. The total thrombus area on the porcine aorta was then quantified using the Applied Imaging Ariol system.

Results: PSI-697 significantly reduced total thrombus area over the denuded porcine aorta in a dose dependant fashion in both the high shear and low shear chambers ($p < 0.05$ in low shear, $p < 0.005$ in high shear), compared to saline. With the higher dosage, there was a greater than 40% reduction in thrombus area in the high shear chamber and an 18% reduction in the low shear chamber. This is due to the fact that the predominant composition of thrombus in the high shear chamber is platelets and not fibrin.



P=significant for saline vs tirofiban,
saline vs PSI low dose,
saline vs PSI high dose

Paired t test, n=12

Conclusion: We have shown for the first time that the novel P-Selectin inhibitor, PSI-697, reduces thrombus load in this model and further establishes the importance of P-Selectin inhibition in prevention of atherothrombotic events.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORγ INHIBITS ANGIOGENESIS BY SUPPRESSING CREB-MEDIATED CYCLOOXYGENASE-2 EXPRESSION IN HUMAN ENDOTHELIUM

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Introduction: Neoangiogenesis contributes to diabetic vasculopathy and intraplaque hemorrhage in atherosclerosis. The activation of Peroxisome Proliferator-Activated Receptor(PPAR)γ is known to inhibit angiogenesis. We therefore aimed at examining the effects of PPARγ agonists on the pro-angiogenic enzyme cyclooxygenase(COX)-2 in human umbilical vein endothelial cells challenged with vascular endothelial growth factor (VEGF) and phorbol 12-myristate 13-acetate (PMA).

Methods and Results: A 24 h exposure of HUVEC to the PPARγ agonists rosiglitazone (RSG) and GW1929 significantly attenuated VEGF- and PMA-stimulated COX-2 activity (by 30%, immunoassay for 6-keto-PGF1α), as well as protein (by 50%, Western analysis) and mRNA expression (by 50%, RT-PCR). This effect was abolished by the PPARγ antagonists bisphenol A diglycidyl ether and GW9662. COX-2 promoter activity experiments revealed that the induction of COX-2 promoter was significantly inhibited by RSG through an interference with the cAMP response element (CRE) site. COX-2 downregulation after siRNA knockdown of the transcription factor CRE binding protein (CREB) confirmed the role of CREB in mediating COX-2 transcription. Correspondingly, PPARγ agonists also attenuated CREB phosphorylation/activation. Since Protein Kinase(PK)C is involved in VEGF-induced COX-2 expression and CREB activation, we also investigated which isoforms of PKC were affected by RSG. While the inhibition of both conventional PKCα and β suppressed VEGF- and PMA-stimulated CREB activation and COX-2 expression, RGS only reduced VEGF- and PMA-stimulated PKCα membrane translocation.

Conclusions: The anti-angiogenic effect of PPARγ agonists is due, at least in part, to their interference with the PKCα-mediated activation of CREB and the related expression of COX-2. PKCα may therefore be a novel therapeutic target for antidiabetic drugs in atherosclerosis.

mCRP ENHANCES PLATELET DEPOSITION AND THROMBUS GROWTH UNDER FLOW; OPPOSING EFFECTS OF CRP ISOFORMS ON THROMBUS GROWTH

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Background and Objectives: Controversial effects of C-reactive protein (CRP) on thrombosis have been reported. However, CRP bioactivity seems based on loss of its pentameric symmetry (natCRP), resulting in formation of modified CRP (mCRP). Our objective was to study the impact of native and modified CRP isoforms on platelet adhesion and thrombus growth under flow conditions.

Methods: Heparinized blood was incubated with either natCRP or mCRP at different concentrations (0, 1 or 5µg/ml) and *in vitro* perfusions were carried out over collagen type I-coated slides using a flat perfusion chamber at 1500 s^{-1}. Images of platelet deposition (mepacrine labeled) were visualized by confocal inverted laser microscope (Leica TCS SP2). Thrombus height was calculated acquiring crossectional images. The area covered by platelets and the area of individual thrombi were evaluated using Image 1.61 Software. Additionally, collagen-coated slides were immunostained after perfusion with anti-CRP antibody to analyze CRP distribution within the thrombus.

Results: mCRP treatment significantly increased platelet deposition (+40 %) and the area of individual thrombi (+50 %), whereas natCRP (5 µg/ml) did not ($p<0.05$). While natCRP did not affect thrombus height, mCRP (5 µg/ml) significantly increased thrombus height (two-fold) ($p<0.002$). CRP protein was distributed within the thrombus and appeared to be differentially expressed in natCRP and mCRP conditions.

Conclusions: Our data indicate that whereas serum natCRP may not affect thrombus growth, mCRP displays a prothrombotic phenotype enhancing not only platelet deposition, but also thrombus growth under arterial flow conditions.

DEVELOPMENT AND VALIDATION OF A RISK SCORE TO PREDICT SERIOUS BLEEDING IN STABLE OUTPATIENTS WITH ATHEROTHROMBOSIS

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Background: In clinical practice, it is often critical to assess the risk of bleeding related to chronic therapy with antiplatelet agents and anticoagulants. A risk score to quantify the bleeding risk for chronic therapy in outpatients would therefore be helpful for clinical decision-making.

Methods: We studied patients enrolled in the REduction of Atherothrombosis for Continued Health (REACH) Registry, an outpatient cohort of 68,375 patients at risk for or with established atherothrombosis. The outcome of interest (studied over a two-year period) was one or more episodes of serious bleeding, defined as bleeding requiring hospitalization and transfusion. Risk factors for bleeding were assessed using multiple regression on bootstrap re-samples. We constructed multiple potential scoring systems based on the least complex models. Competing scores were then compared on their discriminative ability via logistic regression.

Findings: 783 patients had serious bleeding. The final score contained age (1–6 points); peripheral artery disease (2 points); congestive heart failure (2 points); diabetes (1 point); hypertension (2 points); smoking: (3 points); Aspirin (1 point); other antiplatelet agent (2 point); both antiplatelet agents (4 points) and oral anticoagulants (4 points). Risk stratification using our score was effective in classifying the risk level of subjects, with a more than eight-fold increase in risk between the highest and lowest quintiles (cf graphic).

Conclusion: A simple risk score using clinical variables is effective in predicting the risk of serious bleeding related to chronic antithrombotic therapy in outpatients with atherothrombosis.

THROMBIN UP REGULATES EXPRESSION OF ONCOSTATIN M (OSM) IN HUMAN MACROPHAGES AND PERIPHERAL BLOOD MONOCYTES

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Background: Haemostatic factors like thrombin play a crucial role in generating thrombotic plugs at sites of vascular damage (atherothrombosis). However, whether haemostatic factors contribute directly or indirectly to the pathogenesis of atherosclerosis remains uncertain.

Oncostatin M (OSM) as a member of IL-6 family cytokines is a proinflammatory mediator that is primarily known for its effects on cell growth. We could show recently that OSM induces the expression of plasminogen activator inhibitor-1, an established cardiovascular risk factor, in adipose tissue (Rega et al. Circulation 2005). The aim of the present study was to investigate if thrombin and OSM can act as a link between macrophages, platelets and the development of cardiovascular disease.

Methods: Peripheral blood monocytes (PBMC) were isolated using Ficoll-Paque and magnetically labelled CD14 MicroBeads. For macrophage transformation (MDM) cells were cultivated for 8 – 10 days in the presence of human serum. Plaque Macrophages were isolated from atherosclerotic plaques and positive selection of CD14 positive cells was performed employing CD14 antibodies. All Cells were incubated with thrombin at a concentration of 1U/ml. OSM antigen was determined by specific ELISA. OSM specific mRNA was quantitated by RealTime-PCR.

Results: Thrombin increased OSM antigen concentration- and time-dependently up to 20-fold in MDM and up to 8-fold in PBMC. These results could be confirmed on specific mRNA level. In human plaque macrophages stimulation with thrombin leads to a 5-fold increase of OSM mRNA level.

Conclusions: Thrombin induces the expression of OSM in human macrophages in vitro. If this effect is also present in vivo it may be a new link between platelets, macrophages and the development of cardiovascular disease.

INFLUENCE OF CALU A29809G POLYMORPHISM IN ATHEROMA PLAQUE CALCIFICATION: PROGNOSTIC IMPLICATIONS

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It is recognised a pivotal role of vitamin K on calcification homeostasis. In atheroma plaque, activated platelets release different mediators, one of them is calumenin (CALU). CALU is a chaperon of endoplasmic reticulum, and a protein family that joints to Ca^{2+} and inhibits the activity of vitamin K epoxide reductase 1 (VKORC1). It has been proposed that a change a29809g in CALU gene impairs the inhibition of VKORC1, modifying g-carboxilation of vitamin-K proteins, especially matrix Gla proteins. This could also take influence on interstitial calcification. The objectives were to analyse the role of CALU a29809g polymorphism in the calcification of coronary arteries, exploring its functional paper and prognostic implications.

Methods. We studied 222 consecutive patients with non ST elevation acute coronary syndrome. We assessed calcification severity in 165 angiographies of coronary arteries from these patients. We assessed calcification severity giving 0 to 3 in each main arteries (left main artery, left anterior descending artery, circumflex and right coronary artery). Total coronary calcification score was calculated adding all the values in every coronary artery. The presence of CALU a29809g polymorphism was determined by TaqMan assays. TIMI risk score was calculated in all patients. Clinical follow-up at 6 months was performed for adverse endpoints (cardiovascular death, recurrent ACS, revascularization and heart failure).

Results. The frequency of 29809g carriers was 70% (94 ag y 22 gg). 29809g carriers showed more frequently low calcification score in the coronary angiographies (total score 0-1), 80 vs 62% (p=0.028). This association was also observed after adjusting by age, sex and cardiovascular risk factors (p=0.018). Finally, after adjusting by TIMI risk score, the presence of 29809g alelle was associated with a better prognosis at 6 months' follow-up (HR 0.46; p=0.024), whereas TIMI risk score was associated with more adverse events (HR 1.25; p=0.008). Calcification score was not associated with TIMI risk score or prognosis.

Conclusions. The results of the present study demonstrate the role of CALU in the atherothrombotic process, and suggest that CALU a29809g polymorphism could modify g-carboxilation of vitamin-K proteins. CALU a29809g polymorphism seems to be associated with lower calcification of coronary arteries in patients with non ST elevation acute coronary syndrome, and improves significantly their prognosis.

THE METABOLIC SYNDROME IS HIGHLY ASSOCIATED WITH INFLAMMATORY MARKERS AND ADIPOKINES IN ELDERLY MEN

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Purpose. Inflammation plays a central role in the metabolic syndrome (MetS), and adipose tissue is considered an active endocrine organ that releases a large number of adipokines. The objective was to investigate the association between circulating inflammatory markers, adipokines and components of MetS and a potential role of these markers as predictors of cardiovascular events in subjects with and without MetS.

Methods. This was a post hoc analysis from the Diet and Omega-3 Intervention Trial (DOIT), comprising 563 elderly men with (n=221) and without (n=342) MetS.

Results. Several pro-inflammatory markers were elevated in subjects with MetS. Adiponectin, PAI-1 and IL-18 were associated with an increasing number of components of MetS, as well as with most of the MetS components (waist circumference, triglycerides and HDL-cholesterol). During 3 years, 68 cardiovascular events were recorded. In subjects with MetS, CRP (adjusted odds ratio (OR) 3.3 [95% CI 1.1, 10.0]) and IL-18 (adjusted OR 2.7 [1.0, 7.5]) were independent predictors of events. In subjects without MetS, only CRP was an independent predictor. There was a significant interaction between fasting glucose and IL-18 in the cardiovascular risk prediction (p=0.013). Elevated glucose markedly increased the predictive power of inflammatory markers (IL-18: adjusted OR 4.7 [1.2, 18.3], CRP: adjusted OR 5.2 [1.3, 20.0). For IL-18, there was a stepwise increase in ORs by quartiles of glucose.

Conclusions. The components of MetS were highly associated with several adipokines, supporting the hypothesis of adipose tissue as a source of inflammation. Furthermore, we demonstrate for the first time that IL-18 is a predictor of cardiovascular events in subjects with MetS. Moreover, our findings are consistent with a synergistic effect of IL-18 and hyperglycaemia in cardiovascular risk prediction.

Key words: adipokines, IL-18, metabolic syndrome

SHORT-TERM MYOCARDIAL ISCHEMIA INDUCES CARDIAC mCRP EXPRESSION AND PRO-INFLAMMATORY GENE (Cox-2, MCP-1, and TF) UP-REGULATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Background: Prompt coronary thrombus resolution, reducing time of ischemia, improves cardiac recovery. The factors triggered by ischemia that contribute to the clinical outcome are not fully known. We hypothesize that unabated inflammation due to cardiac ischemia may be a contributing factor.

Aims: As a proof-of-concept we evaluated the effect of short-term myocardial ischemia on the local and systemic inflammatory response.

Methods: Pigs either underwent 90 min mid-LAD balloon occlusion (infarct size 25±1% left ventricle; 29% heart function deterioration) or a sham-operation procedure. Peri-infarcted and non-ischemic cardiac tissue was obtained for histopathological, molecular, and immunohistochemical analysis of inflammatory markers (IL-6, TNF-alpha, mCRP, and HAM-56). Blood (femoral vein) was withdrawn prior MI-induction (t=0) and at 30 and 90min to evaluate: 1) systemic cytokine levels (IL-6, TNF-alpha, CRP; 2) pro-inflammatory gene and protein expression in peripheral blood mononuclear cells (PBMC) of TF, Cox-2, MCP-1, and CRP; and 3) platelet activation (assessed by perfusion studies and RhoA activation).

Results: Short-term ischemia triggered cardiac IL-6 and TNF-alpha expression, recruitment of inflammatory cells, and mCRP expression in infiltrated macrophages ($p<0.05$ vs t=0 and sham-operated animals). PBMC mRNA and protein expression of MCP-1, Cox-2, and TF was significantly increased by ischemia whereas no differences were detected in CRP levels. Ischemia increased cTroponin-I, IL-6 and TNF-alpha systemic levels and was associated with higher platelet deposition and RhoA activation ($P<0.001$ vs t=0 and sham).

Conclusion: Short-term myocardial ischemia, even without atherosclerosis, induces an inflammatory phenotype by inducing local recruitment of macrophages and a systemic activation of mononuclear cells and renders platelets more susceptible to activation.

DOSE-EFFECT OF CLOPIDOGREL RE-LOADING IN PATIENTS ALREADY ON 75mg MAINTENANCE DOSE: THE RELOAD STUDY

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Background Clopidogrel loading has mostly been studied in clopidogrel-naïve patients. Whether clopidogrel-treated patients re-admitted for an ACS or a PCI can benefit from a new load of clopidogrel and at what dose remains unknown.

Aim To evaluate the impact of 3 different strategies of administration of a loading dose of 900 mg of clopidogrel in patients already treated with a maintenance dose of 75 mg of clopidogrel for at least 7 days on residual platelet aggregation.

Material and methods Patients chronically treated by clopidogrel 75 mg/day were assigned to receive a first loading dose of 300 mg, 600mg or 900mg of clopidogrel and 4 hours later, a second loading dose of 600, 300 or nothing, respectively, to achieve a total loading dose of 900mg in all patients. Platelet aggregation was evaluated at baseline, 4 hours after the initial load (and before second load) and at 24 hours using light transmission aggregometry with 20µM ADP and the point of care assay VerifyNow®. The primary objective of the study was to evaluate the inhibition (relative change) of residual platelet aggregation (% of IRPA) between 600mg and 900mg first loading at H4. IRPA at 24 hours was also evaluated as a secondary objective as well as the rate of suboptimal response at 4 hours defined as IRPA<10%.

Results: We included 166 consecutive patients with ACS (n=80, 48%) or stable coronary artery disease (n=86, 52%). Baseline characteristics were similar in the three dose groups. There was a significant stepwise increase in % IRPA assessed at 4 hours in patients initially assigned to 300mg vs. 600 mg vs. 900mg (30.7% vs. 40.3% vs. 64.0%, respectively, p=0.0024). The difference in % IRPA at 4 hours was not significant between 300mg and 600mg, but was significant between 600mg and 900mg, as well as between 300mg and 900mg. % IRPA assessed at 24 hours when all patients had received 900mg did not differ between the three loading regimens. The rates of suboptimal response (IRPA<10% at H4) were 23.6%, 20.4% and 5.3%, with 300, 600 and 900 mg, respectively (p=0.02 for all).

Conclusion: In patients chronically treated with 75mg of clopidogrel, a new loading dose of 900mg improves inhibition of PA and reduces poor and/or slow response to clopidogrel, significantly more than what is obtained with 300mg or 600mg.

Key Words: clopidogrel, platelet, inhibition.

POSTERS SESSIONS

POSTERS

Poster Session I

EFFECTS OF ERYTHROCYTES ON PLATELET SIGNAL TRANSDUCTION
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LOW ERYTHROPOIETIN CONCENTRATIONS PREDICT ANGIOGRAPHIC AND ELECTROCARDIOGRAPHIC NO-REFLOW AFTER PRIMARY PERCUTANEOUS CORONARY INTERVENTION
F. Andreotti, G. Niccoli, **F. Marzo**, E. Santucci, C. Spaziani, D. D'Amario, S. Cecchetti, F. Burzotta, F. Crea.
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PROGNOSTIC VALUE OF APOPTOSIS MARKERS IN ADVANCED HEART FAILURE PATIENTS – Panel 3
A. Niessner[1], P.J. Hohensinner[1], K. Rychli[1], S. Neuhold[1], G. Zorn[1], B. Richter[1], M. Hülsmann[1], R. Berger[1], D. Mörtl[1], K. Huber[2], J. Wojta[1], R. Pacher[1].
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IMPACT OF GENETIC POLYMORPHISMS ON PLATELET FUNCTION AND ASPIRIN RESISTANCE
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MODERATE DAILY INTAKE OF RED WINE INHIBITS MURAL THROMBOSIS AND MONOCYTE TISSUE FACTOR EXPRESSION IN AN EXPERIMENTAL PORCINE MODEL
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MODERATE RED WINE INTAKE WITH MEALS REDUCES PLATELET ADHESION AND THROMBOSIS IN NORMOLIPEMIC CONDITIONS.
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A NOVEL CASE OF SELECTIVE ENZYMATIC DEFECT OF CYCLOOXYGENASE-1 ASSOCIATED WITH HAEMORRHAGIC DIATHESIS
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EFFECTS OF ERYTHROCYTES ON PLATELET SIGNAL TRANSDUCTION

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Purpose. Platelet recruitment is a key process in thrombus growth. This is mediated by the release of granule contents (ADP, 5HT, adhesive proteins, etc.) and metabolic products (TXA2) from activated platelets. The agonistic potency of this releasate is strongly increased by cell-cell interactions with metabolically active erythrocytes (RBCs) (1,2).

Aim. To study the participation of receptors for some components of the platelet and platelet-RBC releasate in the signal transduction mechanisms involved in platelet recruitment, particularly those related to protein phosphorylation.

Methods. Washed platelets (WP) or WP+RBCs (Htc. 40%) were stimulated with collagen and rapidly centrifuged to obtain a cell-free releasate within 1 min (1,2). Aliquots of this releasate were used as platelet agonist, and the biochemical effects and proaggregatory action were monitored (recruitment). Tyrosine phosphorylation (TP), cytoskeletal reorganization and cytoskeletal-associated tyrosine phosphorylated proteins were evaluated (3). When appropriate, receptors on recruiting platelets were specifically blocked.

Results. Inhibition of tyrosine kinase activity down-regulated platelet recruitment, while PKC inhibition had no effect. In addition, releasates from WP+RBCs induced high TP levels, cytoskeleton reorganization and translocation of proteins to the cytoskeleton of recruiting platelets. These responses were of comparable final intensity, but even quicker than those induced by 1 U/mL thrombin. Interestingly, RBCs promote a significant translocation of FAK kinase and aIIbb3 to the platelet cytoskeleton, an effect thought to play a role in aIIbb3 clustering and focal adhesion formation. Individual blockade of ADP, TXA2 or serotonin receptors reduced recruitment, TP and cytoskeletal reorganization on recruiting platelets. These reductions were markedly stronger if the ADP receptor P2Y12 or the TXA2 receptor were blocked.

Conclusion. Platelet recruitment constitutes simultaneous signaling events through different receptors that specifically regulate TP and cytoskeletal reorganization. (Grants FIS07/0463; MMA2006; RETICS-RD06/0026). 1- J. Clin Invest 1991; 87: 571-80; 2- Blood 2002; 99: 3978-84; 3- Circulation 2000; 102:1924-30.

Keywords: platelet, erythrocyte, signal transduction

LOW ERYTHROPOIETIN CONCENTRATIONS PREDICT ANGIOGRAPHIC AND ELECTROCARDIOGRAPHIC NO-REFLOW AFTER PRIMARY PERCUTANEOUS CORONARY INTERVENTION

F. Andreotti, G. Niccoli, **F. Marzo**, E. Santucci, C. Spaziani, D. D'Amario, S. Cecchetti, F. Burzotta, F. Crea.
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Aims. No-reflow after percutaneous coronary intervention (PCI) may negate the benefits of coronary patency in patients with acute myocardial infarction (AMI). Models of acute ischemia have shown cardioprotection and attenuated vascular injury by erythropoietin (Epo) through reduced apoptosis, suppressed inflammation, and increased nitric oxide bioavailability. We sought to investigate the association between systemic Epo levels and no-reflow in a group of AMI patients treated by primary PCI.

Methods and Results. From a series of consecutive patients with a first AMI undergoing successful PCI, 48 were investigated (61±12 yrs, 89% male), including the first 24 with angiographic no-reflow and the first 24 without. No-reflow after PCI was defined angiographically (as TIMI flow ≤ 2 or as TIMI flow =3 with a myocardial blush grade ≤ 2) and electrocardiographically (as ≤ 50% resolution of maximal ST elevation). Serum Epo concentrations measured before PCI did not correlate significantly with any baseline characteristic, except white cell count (r=-0.31, p=0.04). Univariate predictors of angiographic and ECG no-reflow were left anterior descending as infarct-related artery (83% vs 29%, p<0.0001, and 75% vs 43%, p=0.04, respectively) and lower Epo levels [median (interquartile): 4.2 (0.6-9.5) vs 12.2 (5.2-20.3) mIU/ml, p=0.001, and 4.0 (0.6-7.1) vs 9.3 (1.0-12.6) mIU/ml, p=0.01, respectively]. At multivariable analysis, only Epo tertiles predicted both angiographic and ECG no-reflow (p=0.008 and p=0.01, respectively).

Conclusions. Epo concentrations showed an independent, graded, and inverse relation with angiographic and ECG no-reflow after successful primary PCI. These data suggest that lower endogenous Epo levels may contribute significantly to coronary microvascular obstruction responsible for no-reflow.

Key words: erythropoietin, acute myocardial infarction, no-reflow.

PROGNOSTIC VALUE OF APOPTOSIS MARKERS IN ADVANCED HEART FAILURE PATIENTS

A. Niessner[1], P.J. Hohensinner[1], K. Rychli[1], S. Neuhold[1], G. Zorn[1], B. Richter[1], M. Hülsmann[1], R. Berger[1], D. Mörtl[1], K. Huber[2], J. Wojta[1], R. Pacher[1].
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Background: The purpose of this study was to assess whether soluble markers of apoptosis predict event-free survival of heart failure patients. Apoptosis plays an important role in progression of heart failure. FAS and TNF-related apoptosis-inducing ligand (TRAIL) induce programmed cell death via separate pathways.

Methods: We assayed soluble (s)FAS and sTRAIL levels in 351 patients with advanced heart failure. During the median follow-up time of 16 months 175 patients (50%) experienced the composite endpoint rehospitalization and death.

Results: The hazard of an adverse event gradually increased with quartiles of sFAS with a hazard ratio (HR) of 2.3 in the highest quartile compared to the lowest quartile. This association remained significant after adjustment for BNP and other known predictors of outcome in a multivariable Cox regression model (P = 0.017). Patients with high sFAS but low BNP had a comparable event-free survival rate to those with elevated BNP only (P = 0.78). Conversely, high sTRAIL concentrations were related to a better prognosis. Particularly, the risk of mortality dropped by 70% in the highest sTRAIL quartile. This inverse correlation remained significant in the multivariable model (P = 0.002).

Conclusions: sFAS is an independent risk predictor of death and rehospitalization in patients with advanced heart failure. It may be of particular value for the identification of high-risk heart failure patients in addition to BNP. Conversely, sTRAIL appears to be protective and could be an interesting therapeutic agent.

IMPACT OF GENETIC POLYMORPHISMS ON PLATELET FUNCTION AND ASPIRIN RESISTANCE

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Purpose: Genetic polymorphisms may affect platelets' responses to the antiplatelet therapy. Our aim was to determine the role of genetic polymorphisms on aspirin resistance in patients with coronary heart disease.

Methods: A total of 126 consecutive patients (35 to 85 years old, 32% women) with chronic stable coronary artery disease (CAD) was enrolled in the study. Platelet function assays were realized by the Platelet Function Analyzer(PFA)-100 with Collagene and epinephrine (Col/Epi) and Collagene and adenosine diphospate (Col/ADP) cartridges. Aspirin resistance was defined as having a closure time (CT) of <186 seconds with Col/Epi cartridges despite regular aspirin therapy. Factor V, prothrombin, factor XIII, β-Fibrinogen, plasminogen activator inhibitor I (PAI-1),glycoprotein IIIa, metylene tetrahydrofolate reductase, ACE and ApoB gene polymophisms were determined by three consecutive step; isolation and amplification of DNA and reverse hibridization.

Results: We determined that 30 patient (23.8%) had aspirin resistance by the PFA-100. Mean closure time measured with the Col/ADP cartridges was 74±12s (51 to 104 seconds). Ten of the 30 subjects with aspirin resistance were women (33.3%). Genetic polymorphisms were determined in 30 aspirin resistant and 17 aspirin sensitive subjects. No statistically significant relationship was determined between aspirin resistance and the genetic panel.

Conclusion: In our study; Factor V, prothrombin, factor XIII, β-Fibrinogen, plasminogen activator inhibitor I (PAI-1), glycoprotein IIIa, metylene tetrahydrofolate reductase, ACE and ApoB gene polymophisms were not correlated with aspirin resistance. Further studies are needed to investigate whether aspirin resistance is related or not with genetic polymorphisms.

Keywords: Aspirin resistance; genetic polymorphisms; coronary heart disease.

MODERATE DAILY INTAKE OF RED WINE INHIBITS MURAL THROMBOSIS AND MONOCYTE TISSUE FACTOR EXPRESSION IN AN EXPERIMENTAL PORCINE MODEL

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Purpose: Moderate consumption of red wine has been epidemiologically associated to a reduction in cardiovascular disease, but its mechanism of action is not fully understood. The objective was to study whether the protective effects of a daily intake of red wine (Tempranillo, 12.8% alcohol v/v) could be related to inhibition of thrombosis in an experimental model of diet induced hyperlipemia.

Methods and Results: Animals were fed, for 100 days, a western-type proatherogenic diet containing 2% of cholesterol and 20% of saturated fat. Three doses of red wine were studied (20-30-40g wine-ethanol/day) and compared with placebo-control animals, not taking any wine. Thrombosis under flow conditions was evaluated by radioisotopic quantitation of deposited platelets on damaged arteries. Changes in RhoA translocation in platelets and monocyte Tissue Factor (TF) expression were also analyzed. Mural platelet deposition was significantly reduced in animals ingesting red wine with their food. Expression of RhoA in the platelet cytoplasm (inactive form) was increased in wine-fed animals. TFmRNA expression in LPS stimulated monocytes was reduced in wine fed animals. Total cholesterol levels were not significantly different among groups.

Conclusions: Moderate red wine intake significantly reduces platelet deposition triggered by damaged vessel wall, partially explained by inhibition of RhoA translocation to the platelet membrane. Hence, a daily moderate intake of wine seems to inhibit different pathways that converge in a reduced thrombotic risk, upon vessel wall injury.

Key words: red wine, thrombosis, cardiovascular disease, tissue factor.

MODERATE RED WINE INTAKE WITH MEALS REDUCES PLATELET ADHESION AND THROMBOSIS IN NORMOLIPEMIC CONDITIONS

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Purpose: As a component of the Mediterranean diet, red wine consumption in moderate amounts has shown to be beneficial against cardiovascular disease. In hyperlipidemia, chronic red wine intake, ingested with food has shown to prevent platelet activation and thrombosis on damaged arterial wall.
The objective was to investigate whether moderate red wine intake inhibits thrombus formation in normolipemic conditions and whether its effects are significant after a single intake or require chronic daily ingestion.

Methods: Two groups of animals were fed for 21days restricted growing chow. One group also received a daily moderate dose of red wine (40g wine ethanol/day) while the other rested as control group. Thrombosis triggered by vascular injury was evaluated in two perfusion chambers. Additionally, platelet aggregation and changes in RhoA translocation, Prostacyclin (PGI_2) release by coronary vessel wall, basal tissue factor (TF) gene-expression in not/induced monocytes, lipid plasma composition and LDL oxidazability (MCD, Maximal Conjugated Diene formation) were analyzed as possible targets of red wine activity.

Results: Blood thrombogenicity, measured as platelet deposition rate (TPD/min of perfusion), was statistically reduced ($P<0.05$) in animals ingesting red wine compared with control animals. Platelet mural deposition was reduced over severely (4,06±0,5 vs. 7,66±1,2 PLT*10^6/cm^2) and mildly (0,66±0,18 vs. 1,54±0,4 PLT*10^6/cm^2) damaged vessel wall in perfusions mimicking blood flow in patent coronary arteries. Platelet adhesion on collagen was also reduced in 56%. Expression of RhoA in the platelet membrane (active form) was statistically reduced in wine-fed animals. PGI_2 was significantly higher (>50%) in coronary tissue of wine fed animals. No differences in plasma lipid composition were observed. LDL oxidation parameters were not significantly modified by red wine intake, MCD= 409,644± 6,4 vs. 429,127± 7,2 nmols/mg protein. In normolipemic conditions, TFmRNA expression in monocytes was not affected in animals ingesting red wine (ratio non-stimulated/LPS stimulated = 12,06 vs. 12,76).

Conclusions: Moderate red wine intake significantly reduces platelet deposition triggered by damaged vessel wall. This inhibitory effect is evident at all levels of vascular damage indicating a general passivation effect of the thrombotic response to injury. Indeed, expression of TF, plasma lipid composition and LDL oxidation were lightly reduced in animals ingesting red wine while vascular PGI_2 production was significantly increased. Platelet RhoA translocation was reduced in animals ingesting wine, suggesting a reduction in platelet activity and a passivating effect in platelet mediated thrombogenicity. Hence the daily wine intake, even in normocholesterolemic conditions, reduces thrombotic risk and induces vascular protection.

Key words: red wine, thrombosis, cardiovascular disease.

A NOVEL CASE OF SELECTIVE ENZYMATIC DEFECT OF CYCLOOXYGENASE-1 ASSOCIATED WITH HAEMORRHAGIC DIATHESIS

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Thromboxane (TX) A_2 is synthesized in activated platelets through the sequential activity of cyclooxygenase (COX)-1 and TXA_2 synthase, and it is a pro-aggregating and vasoconstrictive agent. The importance of this pathway in haemostasis is demonstrated by the efficacy of COX-1 inhibition by aspirin in the prevention of atherothrombotic disorders.

We have studied a 50-year-old woman with a lifelong bleeding history: she had haemorrhagic complications following tonsillectomy, labor, knee surgery, metrorrhagias, appendicectomy and spontaneous epistaxis. Her platelet count was within the normal range ($320x10^3$/mL). She had normal platelet aggregation in response to 5 mM ADP, 4mg/ml collagen and 20 mM epinephrine. However, on two different occasions, she showed a severe defect of platelet aggregation induced by 1.3 mM arachidonic acid: 6% transmittance (N.V. 72-92%). Her bleeding time was 4.30 min (N.V. up to 8 min). COX-1 enzymatic activity, measured by serum TXB_2 released during whole blood clotting in vitro, was repeatedly and severely reduced: 11.58 and 19 ng/ml serum on two different measurements (normal values 200-400 ng/ml serum). The characterization of COX-1 protein was performed by western blot analysis and flow cytometry. Flow cytometry detected a reduction in the mean fluorescent intensity of platelet stained for COX-1 of approx. 30% as compared to normal subjects. Western blotting showed an apparent shift of the molecular weight of COX-1 (approx. 80 kDa, controls approx. 72 kDa), and a reduced expression when normalized to beta-actin. Platelet antigenic levels of CD61 or COX-2 were normal. She referred suffering from chronic gastritis since the age of 12, and erosions on the gastroduodenal mucosa.

Her daughter showed a far less pronounced bleeding diathesis, without major bleeding episodes or methrorrhagies. She also referred dyspeptic symptoms. Her serum TXB_2 levels were 126 ng/ml.
Thus, the proposita showed a combined quantitative and qualitative defect of COX-1 in platelets. Genotyping of the whole family kindred will help identifying whether the defect is transcriptional or post-transcriptional.

Keywords: aspirin, cyclooxygenase, platelet aggregation.

Poster Session II

INCREASING DOSE OF CLOPIDOGREL HAVE NO BENEFIT AMONG PATIENTS RESISTANT TO BOTH CLOPIDOGREL AND ASPIRIN
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PLATELET FUNCTION EVALUATED BY VERIFYNOW® AND P-SELECTIN IN ASPIRIN-TREATED PATIENTS WITH CORONARY ARTERY DISEASE
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DOES A RESIDUAL PLATELET FUNCTION EXIST AFTER DUAL PLATELET THERAPY?
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ANTITHROMBOTIC THERAPY IN PATIENTS TREATED WITH ORAL ANTICOAGULATION UNDERGOING CORONARY STENTING. IMPLICATIONS FOR BLEEDING AND THROMBOSIS RISK.
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PLATELET CYCLOOXYGENASE INHIBITION BY LOW-DOSE ASPIRIN IS NOT REFLECTED CONSISTENTLY BY PLATELET FUNCTION ASSAYS. IMPLICATIONS FOR ASPIRIN "RESISTANCE"
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EFFECT OF CIGARETTE SMOKING ON PLATELET AGGREGATION AND ASPIRIN RESISTANCE
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DETERMINANTS OF PLATELET ACTIVATION IN HEART FAILURE
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PROTEOMIC PROFILE OF MICROPARTICLES DERIVED FROM ACTIVATED PLATELETS
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INCREASING DOSE OF CLOPIDOGREL HAVE NO BENEFIT AMONG PATIENTS RESISTANT TO BOTH CLOPIDOGREL AND ASPIRIN

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Purpose: The variable response to clopidogrel and aspirin among patients undregoing percutaneous coronary intrevention (PCI) has been documented in many recent studies, but the evidences and solutions for simultaneous failure to both drugs are unclear.
In our reaserch,the purpose was to determine if increasing dose of clopidogrel to 150 mg per day have effect on resistance to both clopidogrel and aspirin.

Methods: Our study included elective PCI patients (n=103) who received aspirin 100mg per day ≥ two weeks and clopidogrel 75mg per day was administrated ≥ a week before PCI.
To all (103) patients were perfomed VASP assay to evaluated response to clopidogrel at the 7th day of chronic clopidogrel terapy and Platelet function analzyer test (PFA 100) was performed before introduction of clopidogrel to 60 patients, when patient received only aspirin monotherapy. Aspirin resistance was defined as closure time on PFA100 /CT ≤ 170s, and clopidogrel resistance was defined as platelet reactivity index (PRI) ≥50%. Clopidogrel resistance patients were administrated 150mg of clopidogrel per day in futher 7 days after PCI end VASP assay was performed in third group again to evaluate response to double dose of clopidogrel.

Results: In whole study population there were tventy eight (27,2%) patients resistant to clopidogrel. But in the subgroup tested to both aspirin and clopidogrel (60 patients) there were twenty three patients (38,3%) resistant to aspirin and eighteen (30%) to clopidogrel. Seven (38,9%) of the aspirin resistant patients were also clopidogrel resistant, p=0,954, p>0,05. Amnog 28 patients resistant to clopidogrel, double dose of 150mg clopidogrel per day made 13 patients (46,5%) good responder, but 15 (53,6%) patients remain resistant after double dose. But, in the subgroup tested to both drugs, there were eighteen patients resistant to clopidogrel, among who 13 of them after 150mg clopidogrel per day change their response to non resistant and 5 remains resitance. Even, four (80,0%) of this double dose clopidogrel resistat patients were resistant to aspirin, too(p=0,047, p <0,05).

Conclusion: Our study suggests that patients resistant to both aspirin and standard dose of clopidogrel have no benefit from increasing dose of clopidogrel to 150mg per day.

Keywords: coronary disease, catheterization, platelet

PLATELET FUNCTION EVALUATED BY VERIFYNOW® AND P-SELECTIN IN ASPIRIN-TREATED PATIENTS WITH CORONARY ARTERY DISEASE

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Background: Aspirin inhibits platelet aggregation and is widely used in the treatment of cardiovascular disease. A high inter-individual variation in aspirin response has been reported and referred to as aspirin low-responsiveness. Patients with inadequate platelet inhibition by aspirin might be at increased risk of future cardiovascular events. This problem may be particularly important in diabetic patients.

Aim: To investigate whether type II diabetic patients with coronary artery disease (CAD) have a reduced biochemical response to aspirin compared with non-diabetic patients with CAD, and to study whether there is a reduced response to aspirin in patients with one or more previous myocardial infarction (MI).

Methods: Aspirin response was evaluated by whole blood platelet aggregation with the point-of-care system VerifyNow® Aspirin. Aspirin low-responders were defined by ≥ 550 Aspirin Reaction Units (ARU). Platelet activation was determined by soluble P-selectin (sP-selectin) using ELISA (R&D Systems). We included 177 patients with stable angiographically verified CAD and one or more previous MIs despite aspirin treatment. Among these, 85 had type II diabetes and 92 had no diabetes. All participants were treated with 75 mg of non-enteric coated aspirin for at least 7 days with optimal verification of compliance prior to blood sampling. Analysis of data was performed by two-way ANOVA unless else indicated.

Results: Diabetic patients had a significantly reduced response to aspirin compared with non-diabetic patients (mean ARU: 449±4 vs. 438±3; p=0.03). Diabetic patients had a significant increased level of sP-selectin compared with non-diabetic patients (mean: 78 ±25 vs.66 ±28; p=0.0053). Females had a significantly lower response to aspirin compared with males (mean ARU: 454±7 vs. 441±3; p<0.05). According to the VerifyNow® cut-off level, three diabetic patients were aspirin low-responders compared with one non-diabetic patient (4% vs. 1%; p=0.35; Fisher´s exact test). Using VerifyNow® there was not a reduced response to aspirin in patients with one or more previous MIs (mean ARU; CAD: 445±5 vs. 1 MI: 442±4 vs. >1 MI 443±3; p = 0.93) and also no difference in sP-selectin levels (mean: CAD: 71±28 vs. 1 MI 74±28 vs. >1 MI 70 ±26; p=0.73).

Conclusions: Patients with type II diabetes had a reduced platelet response to aspirin compared with non-diabetic patients. Further studies are needed to determine the risk of future cardiovascular events in patients with inadequate platelet inhibition by aspirin.

Keywords: Aspirin, Platelets, Diabetes Mellitus

DOES A RESIDUAL PLATELET FUNCTION EXIST AFTER DUAL PLATELET THERAPY ?

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A high percentage of patients with symptomatic atherosclerosis still suffer from a serious vascular event despite antiplatelet therapy. It has been proposed different underlying mechanisms, but an incomplete platelet inhibition could take some significant influence. Indeed, this fact has been hypothesised to be involved in late thrombosis of drug eluting stents. Different methods can explore ex vivo response to antiplatelet therapy, although there is no method recognised as a gold-standard. The objective of the present study was to explore the residual platelet function by different laboratory assays in patients under dual antiplatelet therapy. We explored the utility of these findings in a group of patients with late thrombosis of drug eluting stent.

Methods. We included 29 patients with drug eluting stent (14 with late thrombosis, and 15 without any event in the last 12 months), all of them under dual antiplatelet therapy and without any clinical instability in the last 6 weeks. These patients were compared with 45 healthy controls without antitrombotic therapy. Laboratory assays performed in blood were: PFA-100 (Coll-Epi and Coll-ADP), IMPACT (screening test, response to arachidonic acid [AA] and ADP); and VASP phosphorylation (flow cytometric). We also assessed the platelet aggregability in rich platelet plasma with different agonists: AA. ADP, epinephrine, collagen, ristocetin and TRAP-6 amide. We arbitrary considered patients with an incomplete inhibition those with laboratory assays within mean ± 3SD of controls.

Results. All the test, excepting platelet aggregation with ristocetin and IMPACT screening, showed significant differences between patients and controls ($p<0.0001$), demonstrating a clear antiplatelet effect. However, the patients maintained a variable residual platelet function, with a wide range between the different tests. We found an incomplete inhibition: 7% by platelet aggregation with AA and ADP; 30% by IMPACT AA and ADP; 50% by VASP and PFA-100 Coll-Epi; and 76% by PFA-100 Coll-ADP. We were not able to find any significant difference in residual platelet aggregation between patients with or without late thrombosis of drug eluting stent.

Conclusion. We found a frequent residual platelet function in patients under dual antiplatelet therapy. This fact could participate in the recurrence of thrombotic events. Prospective studies will probably identify those laboratory assays useful in the prognostic stratification.

ANTITHROMBOTIC THERAPY IN PATIENTS TREATED WITH ORAL ANTICOAGULATION UNDERGOING CORONARY STENTING. IMPLICATIONS FOR BLEEDING AND THROMBOSIS RISK

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Approximately 10% of all patients requiring a percutaneous coronary intervention (PCI) have an indication for anticoagulation and the optimal antithrombotic strategy is unclear for patients in whom long-term anticoagulation (AC) with vitamin K antagonists is recommended. The aim of this study was to evaluate the antithrombotic treatment after coronary stenting in patients requiring long-term anticoagulation.

Methods: We reviewed consecutive patients with indication for oral anticoagulation undergoing PCI between 2006 to 2008. We recorded clinical characteristics of the patients, cardiovascular risk factors and antithrombotic therapy use before PCI and at discharge. We compared patients that received triple therapy (TT), which included aspirin, clopidogrel and acenocoumarol) against other regimes (non-TT) after PCI. Clinical follow-up was performed, and all bleeding episodes, thromboembolism and stent thrombosis were recorded.

Results: We studied 200 patients (mean age 70±9 years, 72% men). Stent dispositive was implanted in 164, and drug eluting stent (DES) in 59 (36%). Atrial fibrillation (AF) was the main indication for AC treatment (87%). Clinical follow-up was three months since discharge. Of the drugs prescribed at discharge, 103 patients (51%) received triple therapy (coumarins, aspirin and clopidogrel). All cause mortality was 5.5% (4.5% by thrombotic cause). In a multivariate analysis, non-anticoagulation with coumarins increased mortality (hazard ratio [HR]=5.67; p=0.006) and thrombotic events at three months (HR=2.93; p=0.02). Incidence of bleeding was significantly higher in TT-group (HR=3.26; p=0.02), but in a Cox regression analysis there was no significant increase in mortality by major hemorrhagic events in the patients treated with coumarins.

Conclusion: In patients with oral AC indication undergoing PCI with stenting, treatment with coumarins at discharge shows a beneficial effect on prognosis by reducing the incidence of thrombosis and death. TT predisposes to an increased risk of bleeding, however these complications do not appear to be associated with a substantial increase in mortality.

PLATELET CYCLOOXYGENASE INHIBITION BY LOW-DOSE ASPIRIN IS NOT REFLECTED CONSISTENTLY BY PLATELET FUNCTION ASSAYS. IMPLICATIONS FOR ASPIRIN "RESISTANCE"

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Purpose: Functional assays of the antiplatelet effects of low-dose aspirin variably reflect the thromboxane-dependent component of platelet aggregation. We assessed the thromboxane-dependence of biochemical and functional indexes used to monitor the effect of low-dose aspirin, and the inter- and intra-subject variability on aspirin and after its withdrawal.

Methods: Forty-eight healthy volunteers were randomized to receive aspirin 100 mg/od for one to eight weeks.

Results: Serum thromboxane (TX)B_2 was evenly suppressed by ≥99%. Urinary 11-dehydro-TXB_2, arachidonic acid-induced aggregation and Verify-Now-Aspirin® showed a stable, incomplete inhibition (65, 80 and 35%, respectively). Adenosine-diphosphate- and collagen-induced aggregation were highly variable, poorly aspirin-sensitive, with an apparent time-dependent reversal. Inhibition of TXB_2 was non-linearly related to aggregation inhibition. Platelet function largely recovered by day 3 post-aspirin, independently of treatment duration. With any functional assay, occasionally "resistant" subjects were always "responder" on previous or subsequent determinations.

Conclusion: Platelet cyclooxygenase activity, as reflected by serum TXB_2, is uniformly and persistently suppressed by low-dose aspirin in healthy subjects. However, the effect of aspirin is variably and randomly detected by functional assays, potentially leading to misclassification of "responder" and "resistant" phenotype due to their poor reproducibility. The non-linear relationship between inhibition of TXB_2 production versus platelet function has important clinical implications.

Keywords: antiplatelet drugs, thromboxanes, cyclooxygenase

EFFECT OF CIGARETTE SMOKING ON PLATELET AGGREGATION AND ASPIRIN RESISTANCE

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Purpose: Cigarette smoking may increase platelet aggregation and cause atherothrombotic cardiovascular events. We aimed to investigate the role of cigarette smoking on platelet function in patients with stable coronary heart disease.

Methods: Twenty patients with stable coronary heart disease under aspirin therapy (300 mg daily) who did not leave cigarette smoking despite all warnings was enrolled in the study. The study was approved by local ethics committee. We studied platelet function of the subjects in the morning; before and fifteen minutes after the first cigarette smoke. We determined platelet function by the 'Platelet Function Analyzer(PFA)-100' device with collagen and epinephrine (col/epi) and collagen and adenosin diphosphate (col/adp) cartridges. We accepted patients who had a closure time shorter than 186s despite regular aspirin therapy as aspirin resistant subjects.

Results: Aspirin resistance was present in four of the enrolled subjects (20%) at the beginning. We analyzed the blood samples obtained fifteen minutes after cigarette smoking. We determined a significant increase in platelet aggregation by the PFA-100 after a single cigarette smoking (p=0.001). We established that closure time was also shortened after cigarette smoking in patients with aspirin resistance. Four more patients became aspirin resistant after a single cigarette smoking (p=0.004). We did not determine significant differences in demographic, hematological and biochemical parameters between the aspirin resistant and sensitive subjects.

Conclusion: We determined that even a single cigarette smoking may increase platelet aggregation both in aspirin resistant and sensitive patients with coronary heart disease. We also determined that aspirin therapy failed to prevent the increase of platelet aggregation caused by cigarette smoking. Our findings emphasize also the importance of leaving cigarette smoking in patients with coronary heart disease.

Variable	Aspirin resistant	Aspirin sensitive	p
Age	49 ± 6	33 ± 6	<0.001
Gender (male)	4	15	0.378
Hypertension	3	9	0.619
Diabetes mellitus	0	0	NS
Aspirin dose (mg)	300	300	NS
Platelet count	167 000	157 000	0.263
Creatinin	1,02	0,98	0.476
COL/ADP	88 ± 6	86 ± 4	0.523

Keywords: Aspirin resistance; cigarette smoking; coronary heart disease.

DETERMINANTS OF PLATELET ACTIVATION IN HEART FAILURE

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Purpose: Thromboembolism is a critical and relatively common complication of chronic heart failure (HF).

Methods: We performed a cross-sectional study in 84 HF patients [33 M; 81±8 yr; 49 in I-II, 35 in III-IV New York Heart Association (NYHA) class] and 42 controls, using urinary (U) 8-iso-prostaglandin (PG) F_2a and 11-dehydro-thromboxane (TX) B_2 as non-invasive indexes of oxidative stress and platelet activation, respectively, B-type natriuretic peptide (BNP) as a biomarker of cardiac function, plasma asymmetric dimethylarginine (ADMA) as an index of endothelial dysfunction, C-reactive protein (CRP) and sCD40 ligand (sCD40L) as markers of inflammation.

Results: Forty-two HF patients not on aspirin treatment had significantly higher U-11-dehydro-TXB_2 excretion [Median (IQR): 1488(824-2130) vs 440(313-611) pg/mg cr], 8-iso-PGF_2a [528(430-702) vs. 304(228-364) pg/mg cr], BNP [363(196-659) vs 78(56-98) pg/mL], ADMA (1.6±0.5 vs 0.5±0.2 mmol/L), CRP [1.74(0.98-2.7) vs 0.5(0.4-0.7) mg/L] and sCD40L levels [1342(653-2320) vs 432(322-840) pg/mL] (all $p<0.0001$) than controls. Forty-two HF patients on low-dose aspirin showed significantly lower 11-dehydro-TXB_2 [343(227-455) pg/mg cr, $p<0.007$] and sCD40L levels [820(535-1160) pg/mL, $p<0.02$] than HF patients not on aspirin. Patients in NYHA classes III-IV showed higher U-11-dehydro-TXB_2 excretion than patients in I-II classes, independently of aspirin treatment ($p<0.05$). In the 42 HF patients not on aspirin, U-11-dehydro-TXB_2 was correlated with BNP (Rs=0.59), 8-iso-PGF_2a (Rs=0.58), and CD40L (Rs=0.61) (all $p<0.0001$). Multiple regression analysis revealed that higher BNP levels (Beta Coefficient=0.74), no aspirin therapy (-0.41), and higher sCD40L levels (0.32) (all $p<0.0001$), independently predicted the excretion rate of 11-dehydro-TXB_2 in the 84 pts.

Conclusion: Persistent platelet activation characterizes patients with heart failure. This phenomenon is related to disease severity and is largely suppressable by low-dose aspirin.

Keywords: Heart failure, thromboxanes, platelets.

PROTEOMIC PROFILE OF MICROPARTICLES DERIVED FROM ACTIVATED PLATELETS

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Introduction: There is increasing evidence that platelet microparticles (MPs) participate in thrombus formation. Besides, its role in thrombosis, MPs are believed to mediate different processes involved in atherosclerosis since they convey plasma membrane and cytoplasmic components present at the vicinity of their shedding sites. However, up to now protein composition of microparticles derived from activated platelets remain poorly defined. In the present study, our goals were (1) to study the proteomic profile of MPs derived from thrombin-activated platelets and (2) to identify differential proteins related to platelet function and thrombogenicity.

Methods: MPs were prepared from a suspension of human washed platelets ($4x10^5$/μL) obtained from healthy donors and activated in vitro with 0.5 uNIH/mL thrombin, during 3 min at 37ºC. Platelets and platelet derived MPs were separated by centrifugation (3200g, 15min). MPs were isolated from the platelet-free secretome by ultracentrifugation and characterized by flow cytometry, using annexin V and CD41. Proteins were extracted by a Tris-triton-X100 buffer. Proteomic studies were performed by bidimensional electrophoresis (2DE) and mass spectrometry (MALDI-ToF). Proteins were identified using Swiss-Prot database. Differences in the protein patterns were analysed using specific data analysis software (PDQuest). Identified differential proteins were validated by standard western blot.

Results: 2DE electrophoresis has shown that platelet MPs displayed 385±14 different protein features (characterized by pI and MW, 3 independent experiments). Identification of proteins by MALDI-TOF analysis revealed a high percentage of proteins related to the cytoskeleton and involved in cytokinesis (27.1%), cell surface (13.6%), and signalling processes (22.1%). The intensity level of 37% of the protein spots was more than 2fold modified when MPs derived from thrombin activated platelets. Modifications included newly detected spots (7), lost of detection (13), increased detection (89), and decreased detection (43). Protein changes related mainly to cytoskeleton associated proteins (MRLC, gelsolin), membrane surface proteins (gp140, GPIIb, thrombospondin). Also proteins as PDI and the heat shock protein 70 (BiP) that according with our previous studies play a role in activated platelets were detected and presented a differential pattern in activated platelet derived MP.

Conclusion: Activated platelet derived microparticles depicted a proteomic profile that strongly supports their functional involvement in atherothrombosis. In addition, proteins identified in the present study might bring new clues on the mechanisms involved in MP formation and function in cardiovascular diseases.

Key words: Platelets, microparticles, proteomics

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