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Habilitation Thesis

Vascular Inflammation in Coronary Artery Disease:

Pathophysiological Pathways and Clinical Implications

to obtain a lecture qualification for the profession of internal medicine and cardiology

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by

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ABBREVIATIONS

CABG	Coronary artery bypass graft
CAPE	Caffeic acid phenethyl ester
CI	Confidence interval
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
ECG	Electrocardiographic
GRACE	Global Registry of Acute Coronary Events
H-FABP	Heart-type fatty acid binding protein
Hs-cTnT	High-sensitivity cardiac troponin T
KCl	Potassium chloride
LDL	Low-density lipoprotein
L-NAME	N^{ω}-nitro-L-arginine methyl ester
MAP kinase	Mitogen-activated protein kinase
MRP 8/14	Myeloid-related protein 8/14
NAD	Nicotinamide adenine dinucleotide
Na^+/K^+-ATPase	Sodium-potassium-activated adenosine triphosphatase
NO	Nitric oxide
OR	Odds ratio
PAPP-A	Pregnancy-associated plasma protein-A
PARP-1	Poly(adenosine diphosphate [ADP]-ribose) polymerase-1
PCI	Percutaneous coronary intervention
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PI3K	Phosphoinositide 3-kinase

RNA	Ribonucleic acid
ROS	Reactive oxygen species
SVG	Saphenous vein graft
TIMI	Thrombolysis in Myocardial Infarction
TNF-α	Tumor necrosis factor alpha

INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality in the Western world,(1) and its prevalence is expected to rise in near future given the increasing burden of obesity and diabetes, along with the ageing of the population. Early identification of patients at increased risk gained therefore further importance, and novel diagnostic and therapeutic approaches to improve patient outcomes are needed. Much research interest is focused on inflammatory processes within the arterial wall which are known to be importantly involved in the pathogenesis of atherosclerosis. The endothelium, by modulating vascular tone, regulating hemostasis, and orchestrating inflammatory cascades, constitutes a key player in the disease process. A better understanding of pathophysiological mechanisms may pave the way for novel therapeutic concepts.

In the present habilitation thesis, aspects of altered endothelial function under inflammatory conditions are discussed with main focus on the regulation of vascular tone and coagulation. Further, potential clinical implications for patients with coronary artery disease are elucidated.

The endothelium in cardiovascular disease

The endothelium as the innermost layer of the arterial wall separates the circulating blood from the vessel wall and maintains vascular integrity and hemostasis. Endothelial factors such as nitric oxide (NO) are importantly involved in maintaining the balance between vasodilation and vasoconstriction, thereby regulating blood flow. Besides acetylcholine, various factors such as histamine, prostaglandins or shear stress can evoke endothelium-dependent relaxation, mainly mediated via NO.(2) Nitric oxide, generated in endothelial cells from the amino acid L-arginine by the NO synthase, induces vascular smooth muscle cell relaxation via guanylate cyclase

activation, and further exerts pleiotropic anti-inflammatory, anti-proliferative, and anti-platelet effects.(2, 3) A functional endothelial layer further prevents thrombus formation and platelet aggregation not only by separating the circulating blood from the highly thrombogenic subendothelial layer, but also by expressing anti-coagulant or fibrinolytic factors such as tissue factor pathway inhibitor or tissue-type plasminogen activator.(4, 5)

Chronic exposure to cardiovascular risk factors such as diabetes, hypertension, obesity, or smoking have been associated with endothelial dysfunction.(2, 6, 7) Activated endothelial cells are characterized by the release of prothrombotic and vasoactive substances such as tissue factor or endothelin-1,(5, 8) and display an enhanced production of reactive oxygen species (ROS), finally resulting in decreased NO bioavailability.(2, 6) When endothelial function is impaired, vasodilator capacities are reduced, and vessels prone to vasospasm. Endothelial dysfunction can be investigated *in vitro* in organ chamber experiments for isometric tension recording, and *in vivo* by different modalities such as the assessment of flow-mediated dilation of the brachial artery or the measurement of coronary blood flow velocities.(9-11) Further, activated endothelial cells display an enhanced expression of adhesion molecules including intercellular adhesion molecule 1, vascular cell adhesion molecule 1 or selectins, and thereby promote leukocyte adhesion and migration.(12) Endothelial dysfunction is therefore considered the initial step in the pathogenesis of atherosclerosis, and an enhanced proliferation of inflammatory and vascular smooth muscle cells, along with the accumulation and peroxidation of lipids, ultimately lead to the formation of atherosclerotic plaques which may cause flow-limiting stenosis or acute plaque rupture.(13)

Tissue factor (coagulation factor III, F3), the initiator of the extrinsic coagulation cascade, is expressed by various vascular cell types including activated endothelial

cells, vascular smooth muscle cells, and monocytes, and can further be detected in the circulating blood in tissue factor containing microparticles and as an alternatively spliced soluble isoform.(14-17) It is well documented that tissue factor is highly expressed in atherosclerotic plaques,(18) and increased in patients with unstable angina and acute myocardial infarction.(19, 20) Tissue factor binds activated factor VII and in turn catalyzes the activation of factor IX and factor X, ultimately leading to fibrin formation and thrombus generation.(21) Tissue factor expression is upregulated by different inflammatory mediators such as thrombin, tumor necrosis factor alpha (TNF-α), lipopolysaccharides, or interleukin-1, (14, 22, 23) as well as by vasoactive amines including histamine and serotonin.(24, 25) These mediators act via various intracellular signal transduction pathways including mitogen-activated protein (MAP) kinases, phosphoinositide 3-kinase (PI3K), or protein kinase C.(14) Alternative splicing of the primary full-length tissue factor gene transcript eliciting a loss of exon 5 results in the formation of the soluble alternatively spliced tissue factor protein form, which lacks the transmembrane domain and is considered to exert less procoagulant activity as compared to the full-length form.(15, 26) Most recently, small non-coding ribonucleic acids (microRNAs) such as microRNA-19b and microRNA-223 have been identified as post-transcriptional regulators of endothelial tissue factor expression and procoagulant activity.(27, 28) Beyond its critical role in coagulation, tissue factor has been shown to modulate inflammatory responses, mostly via interaction with protease-activated receptors.(29) Hence, tissue factor acting as a key player in coagulation and inflammation may represent an interesting target for novel anti-thrombotic as well as anti-inflammatory therapeutic strategies in patients with coronary artery disease.

The adhesion molecule P-selectin, expressed at the surface of activated endothelial cells and platelets, mediates interactions between leukocytes and platelets and the activated vessel wall, thereby acting at the interface between thrombosis and

inflammation.(30, 31) Being stored in α and dense granules of platelets and Weibel-Palade bodies of endothelial cells, P-selectin is rapidly expressed at the cellular surface upon stimulation, and protein expression is further enhanced by various inflammatory cytokines.(30) P-selectin binds to its primary ligand P-selectin glycoprotein ligand-1 that is constitutively expressed on leukocytes, and thereby supports platelet-leukocyte interactions and initiates leukocyte rolling on the endothelium.(30, 32) Given its central role in mediating cell-cell-interactions, P-selectin is a key player in the pathogenesis of both atherosclerosis and thrombosis, and may represent a promising therapeutic target in selected patients with cardiovascular diseases. P-selectin-based therapies are further supported by studies demonstrating reduced neointima formation and in-stent restenosis after inhibition of P-selectin-mediated leukocyte recruitment in animal models of vascular injury,(33, 34) and a smaller infarction size after P-selectin antibody administration in a rat model of myocardial ischemia-reperfusion injury.(35) Further, the recent SELECT-ACS (Effects of the P-Selectin Antagonist Inclacumab on Myocardial Damage After Percutaneous Coronary Intervention for Non-ST-Segment Elevation Myocardial Infarction) trial suggested beneficial effects of the P-selectin antibody inclacumab on peri-procedural myocardial infarction in patients with non-ST-segment elevation acute coronary syndromes undergoing percutaneous coronary intervention (PCI).(36)

Coronary stents and stent thrombosis

In patients with flow-limiting coronary artery disease, revascularization by PCI or coronary artery bypass graft (CABG) surgery is the standard treatment, along with optimal medical therapy, to reduce symptoms and improve patient outcomes.(37) After the introduction of percutaneous coronary revascularization by Andreas Grüntzig in 1977,(38) PCI has become one of the most frequently performed therapeutic

procedures worldwide, and has experienced an impressive refinement with the advent of coronary stents and the use of highly potent anti-platelet agents.(39-43)

Albeit rare and occurring with rates of about 1% at 1 year and 0.2 to 0.4% yearly thereafter,(44) stent thrombosis remains one of the feared complications in patients undergoing coronary artery stent implantation.(45-47) Stent thrombosis mostly presents as ST-segment elevation myocardial infarction and has been associated with high mortality rates of about 20 to 40%.(44, 47) The pathogenesis of stent thrombosis is multifactorial, and patient-, lesion-, and procedure-related factors are importantly involved. Predisposing factors include comorbidities such as diabetes and renal failure, premature cessation of anti-platelet therapy, complex lesion morphology, as well as malapposed, underexpanded and uncovered stent struts.(48-51) In recent years, impressive technical developments including the implementation of novel stent platforms and polymers, along with the use of potent anti-proliferative agents, were made to further advance stent design and improve vascular healing responses. Recent registries reported an improved safety and efficacy with newer-generation drug-eluting stents such as the zotarolimus- and everolimus-eluting stent as compared with first-generation drug-eluting stents with a reduced risk of stent thrombosis.(48, 52, 53) Indeed, in a large registry of unselected patients undergoing coronary artery stenting, the cumulative incidence of definitive stent thrombosis at 3 years was 1.5% with bare metal stents, and 2.2% and 1.0% with first- and second-generation drug-eluting stents.(48) However, similar to rapamycin and paclitaxel used on first-generation drug-eluting stents,(54, 55) both everolimus and zotarolimus were found to exert pro-thrombotic properties and increase tissue factor expression.(56) Hence, alternative compounds combining anti-thrombotic, anti-inflammatory, and anti-proliferative effects may have the potential to further improve patient outcomes following coronary artery stent deployment.

Coronary artery bypass graft surgery and saphenous vein graft disease

Coronary artery bypass graft surgery is one of the most commonly performed surgical procedures and the preferred coronary revascularization strategy in selected patients with severe coronary artery disease including those with left main and three-vessel disease, particularly when the proximal left anterior descending coronary artery is involved and in diabetic patients.(37, 57, 58) In CABG surgery, autologous saphenous vein grafts (SVG) remain the most frequently used graft. However, SVG failure continues to impede outcomes following CABG surgery as vein grafts are often subjected to endothelial damage during harvesting and then chronically exposed to arterial pressures. Hence, they are characterized by reduced patency rates as compared with arterial conduits.(59, 60) Saphenous vein graft disease has been observed in up to 50% of patients at 1 year after surgery in recent studies, and graft patency rates at 10 years are considered to be around 30 to 70%.(37, 59, 61) While thrombosis remains a major cause of vessel occlusion when SVGs fail early after CABG surgery, neointimal hyperplasia and accelerated atherosclerosis typically occur in later stages.(62) Considering these pathophysiological mechanisms, it is not surprising that therapeutic strategies targeting platelet aggregation and lipid accumulation have shown favourable effects on the progression of SVG disease and occlusion.(63-65) While aspirin was clearly associated with improved SVG patency after CABG surgery,(65, 66) dual antiplatelet therapy including aspirin and clopidogrel did not yield incremental benefit.(67) Given the high prevalence of failed vein grafts and the increased mortality associated with redo CABG surgery,(68) along with the limited pharmacological treatment options currently available, there is an unmet clinical need for novel therapeutic concepts in this field. In view of the pivotal role of the adhesion molecule P-selectin in inflammation and thrombosis discussed above, (33, 34, 69) and the observed attenuated leukocyte adherence to mechanically dilated

SVGs after administration of an anti-P-selectin antibody,(69) we hypothesized that P-selectin-directed therapies may exert beneficial effects in SVG disease.

Inflammatory biomarkers in cardiovascular disease risk assessment

As highlighted above, inflammatory cascades represent key pathophysiological mechanisms of atherosclerosis, and based on the concept that inflammatory biomarkers mirror processes within the vessel wall, research interest has focused on the identification of novel markers as mediators of cardiovascular risk in different patient populations. Patients with suspected acute coronary syndromes represent an important patient subset at the emergency department, and rapid identification of patients with true coronary events is crucial and provides the basis for a timely treatment strategy. Besides clinical judgement and electrocardiographic (ECG) changes, cardiac biomarkers complement patient assessment and early risk stratification. In recent years, high-sensitivity cardiac troponins (hs-cTn) have been shown to diagnose acute myocardial infarction at an earlier point in time and with a higher sensitivity as compared to conventional assays, however, at the expense of a decreased specificity.(70-72) Indeed, various other clinical conditions including pulmonary embolism, tachyarrhythmia, hypertension, and sepsis may cause elevated troponin levels, and thereby hamper decision making in clinical practice.(73) Indeed, the positive predictive value of small elevations of hs-cTn in predicting acute coronary syndromes may be low.(70, 72) Therefore, additional rule-in parameters are needed in patients presenting with suspected acute coronary syndromes to identify those at increased risk of adverse events and need for timely coronary revascularization. Various inflammatory biomarkers such as heart-type fatty acid binding protein (H-FABP), myeloperoxidase, or myeloid-related protein (MRP) 8/14 have been suggested in this context,(74, 75) and multimarker testing – in comparison to a single-marker

strategy – was proposed as an attractive tool to improve risk prediction in patients with suspected acute coronary syndromes.(76-78) Major efforts are currently being undertaken to identify novel markers with increased diagnostic accuracy and improved predictive value.

AIMS

The aims of this habilitation thesis were:

1) To investigate the endothelial regulation of vascular tone under inflammatory conditions.
2) To elucidate the endothelial regulation of coagulation under inflammatory conditions and develop novel therapeutic strategies.
3) To investigate the predictive role of inflammatory biomarkers in patients with suspected acute coronary syndromes.

RESULTS

1. **Absence of histamine-induced nitric oxide release in the human radial artery: implications for vasospasm of coronary artery bypass vessels.** Stähli BE,* Greutert H,* Mei S, Graf P, Frischknecht K, Stalder M, Englberger L, Künzli A, Schärer L, Lüscher TF, Carrel TP, Tanner FC. American Journal of Physiology – Heart and Circulatory Physiology 2006;290(3):H1182-9. (*shared first authorship)

The radial artery is known to be prone to vasospasm both when used as bypass graft vessel and during coronary angiography and PCI performed via the radial access.(79, 80) As histamine is known to elicit vasospasm,(81) its effect on vascular reactivity of different vessels used as bypass grafts including the radial artery, the internal mammary artery, and the saphenous vein was compared. Vessel segments were collected from patients undergoing CABG surgery and examined in organ chamber experiments for isometric tension recording. Histamine H_2-receptor expression was assessed by real-time polymerase chain reaction (PCR), and endothelial NO synthase expression by Western blot analysis. This study showed that after precontraction with norepinephrine, histamine at lower concentrations induced relaxations in the internal mammary artery (-31.2±3.7% of contraction to potassium chloride [KCl]) and the saphenous vein (-13.0±3.6% of contraction to KCl), but not in the radial artery. At higher concentrations, histamine-induced contractions reached similar levels in all three vessels (p=ns). Endothelial removal, the competitive antagonist of NO formation N^{ω}-nitro-L-arginine methyl ester (L-NAME), and the histamine H_2-receptor blocker cimetidine blunted relaxations in the internal mammary artery and the saphenous vein ($p<0.05$), but did not alter histamine responses in the radial artery. The cyclooxygenase inhibitor indomethacin enhanced relaxations ($p<0.05$) and tended to reduce contractions ($p=0.10$) to histamine in the saphenous vein. Consistently, a lower

endothelial histamine H_2-receptor expression was detected in the radial artery as compared to the internal mammary artery and the saphenous vein ($p<0.05$), while endothelial NO synthase expression was similar in the three vessels (p=ns). Hence, histamine-induced relaxations of the mammary artery and the saphenous vein appear to be caused by NO release mediated via activation of endothelial H_2-receptors, and the lower endothelial histamine H_2-receptor expression in the radial artery may explain the absence of relaxations in this vessel. Further, as indomethacin enhanced relaxations and reduced contractions in saphenous veins, vasoconstrictor prostaglandins seem to counteract relaxations in vein grafts. These findings illustrate a different regulation of vascular tone among different vascular beds, and may represent a possible mechanism for vasospasm observed in the radial artery.

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2. **Poly(ADP-ribose) polymerase-1 protects from oxidative stress induced endothelial dysfunction.** Gebhard C*, Stähli BE*, Shi Y, Camici GG, Akhmedov A, Hoegger L, Lohmann C, Matter CM, Hassa PO, Hottiger MO, Malinski T, Lüscher TF, Tanner FC. Biochemical and Biophysical Research Communications. 2011;414:641-6. (*shared first authorship)

As highlighted above, endothelial dysfunction occurring in the early phases of atherosclerosis is associated with an increased production of ROS. Poly(adenosine diphosphate [ADP]-ribose) polymerase-1 (PARP-1) is a nuclear chromatin-associated enzyme which transfers ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to itself and other nuclear acceptor proteins, and acts as a downstream effector of oxidative stress.(82, 83) This study aimed at investigating the role of PARP-1 in endothelial dysfunction under conditions of intracellular oxidative stress. Therefore, PARP-1 (-/-) and PARP-1 (+/+) mice were treated with paraquat (10 mg/kg i.p.) to induce oxidative stress,(84) and aortic rings were suspended in organ chambers for isometric tension recording. Paraquat impaired endothelium-dependent relaxations in PARP-1 (-/-) mice, but not in PARP-1 (+/+) mice ($p<0.001$). Paraquat enhanced contractions to norepinephrine by 1.9-fold in PARP-1 (-/-) as compared to PARP-1 (+/+) mice ($p<0.001$). Paraquat-induced alterations of endothelium-dependent relaxation and norepinephrine-induced contractions in PARP-1 (-/-) mice were prevented by polyethylene glycol (PEG)-superoxide dismutase and PEG-catalase, two scavengers of superoxide anion and hydrogen peroxide, as well as by indomethacin. L-NAME caused baseline contractions in paraquat-treated PARP-1 (-/-) mice, and increased acetylcholine-induced contractions by 3.3-fold in paraquat-treated PARP-1 (-/-) mice as compared to PARP-1 (+/+) mice ($p<0.001$), demonstrating that NO bioavailability is preserved under basal and stimulated conditions. The vasoconstrictor

effects of L-NAME were inhibited by indomethacin. Extracellular peroxynitrite and NO concentrations were similar in PARP-1 (-/-) and PARP-1 (+/+) mice. These results suggest that PARP-1 activity protects from oxidative stress-induced endothelial dysfunction by inhibiting the production of vasoconstrictor prostanoids, and thereby may play an important role in maintaining endothelial function under these conditions.

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3. Cardiac glycosides regulate endothelial tissue factor expression in culture.

Stähli BE, Breitenstein A, Akhmedov A, Camici GG, Shojaati K, Bogdanov N, Steffel J, Ringli D, Lüscher TF, Tanner FC. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007;27:2769-76.

Tissue factor is a key regulator of the coagulation cascade, and has been implicated in the pathogenesis of acute coronary syndromes. Stent thrombosis is a dreaded complication following stent implantation given the associated high morbidity and mortality. Hence, much interest is focused on the development of novel stent designs and the search for alternative drugs to attenuate both proliferative and thrombotic responses to vascular injury following stent deployment. Therefore, the aim of this study was to assess the effect of cardiac glycosides, known to impair vascular smooth muscle cell proliferation at higher concentrations,(85) on endothelial tissue factor expression. A concomitant inhibitory effect on tissue factor expression would render cardiac glycosides particularly well suited for the application on drug-eluting stents. We therefore assessed the effect of the cardiac glycoside ouabain on TNF-α-induced tissue factor expression in human aortic endothelial cells. We demonstrated that ouabain significantly reduced TNF-α-induced endothelial tissue factor protein expression with a maximal inhibition of 70% at a concentration of 10^{-5} mol/L ($p<0.001$), and reduced tissue factor surface activity by 44% ($p<0.001$). Ouabain-induced inhibition of the sodium-potassium-activated adenosine triphosphatase (Na^+/K^+-ATPase) activity was confirmed by a ouabain-induced decrease of ^{86}Rb influx ($p<0.05$). Consistently, inhibition of Na^+/K^+-ATPase activity by lowering extracellular potassium concentrations inhibited TNF-α-induced tissue factor protein expression ($p<0.001$). As the gap junction inhibitor carbenoxolon did not alter TNF-α-induced endothelial tissue factor protein expression, ouabain does not exert its effect by interfering with the

function of gap junctions. Further, our results suggest that ouabain effects are mediated at the post-transcriptional level as expression of full-length tissue factor mRNA was not altered, and as ouabain did not affect tissue factor protein degradation. These findings provide novel insights into the regulation of endothelial tissue factor expression, and may open new avenues for potential applications of cardiac glycosides when applied locally, e. g. on drug-eluting stents.

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4. **Caffeic acid phenethyl ester inhibits endothelial tissue factor expression.** Gebhard C,* Stähli BE,* Largiadèr S, Holy EW, Akhmedov A, Camici GG, Lüscher TF, Tanner FC. Biological and Pharmaceutical Bulletin 2013;36(6):1032-35. (*shared first authorship)

As highlighted above, the key role in the coagulation cascade renders tissue factor a promising therapeutic target in cardiovascular disease, and besides cardiac glycosides, we tested other anti-inflammatory compounds for their ability to alter tissue factor expression. Caffeic acid phenethyl ester (CAPE) is an active component of propolis from honeybee hives, which has previously been shown to exert anti-oxidant and anti-inflammatory properties and to inhibit platelet activation.(86, 87) In animal models of vascular injury and atherosclerosis, CAPE prevented restenosis after balloon angioplasty and reduced atherosclerotic plaque formation.(88, 89) Given these beneficial effects of CAPE and the critical role of tissue factor in the coagulation cascade, this study was designed to assess whether CAPE modulates endothelial tissue factor expression. We demonstrated that CAPE significantly reduced TNF-α-induced endothelial tissue factor protein expression reaching a 2.1-fold decrease at 10^{-5} mol/L ($p<0.001$). Consistently, CAPE inhibited tissue factor surface activity ($p=0.02$). Tumor necrosis factor-α-induced MAP kinase activation, tissue factor promoter activity, and tissue factor mRNA expression were not affected by CAPE. CAPE did not alter TNF-α-induced IκB-α degradation, but slightly prolonged its resynthesis as compared to TNF-α alone ($p=0.045$). However, as neither promoter activity nor TF mRNA expression were altered, these alterations in the kinetic profile of IκB-α resynthesis are unlikely to mediate the effects of CAPE on tissue factor expression. Hence, a post-transcriptional regulation of tissue factor expression is again

suggested. Future studies are needed to assess whether CAPE may represent a promising therapeutic compound for patients with cardiovascular disease.

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5. **Effects of P-selectin antagonist inclacumab in patients undergoing coronary artery bypass graft surgery: SELECT-CABG Trial.** Stähli BE, Tardif JC, Carrier M, Gallo R, Emery RW, Robb S, Cournoyer D, Blondeau L, Johnson D, Mann J, Lespérance J, Guertin MC, L. L'Allier P. Journal of the American College of Cardiology. 2016;67(3):344-6.

Besides tissue factor, the adhesion molecule P-selectin is importantly involved in the regulation of hemostasis. P-selectin is expressed on activated endothelial cells and platelets, and stimulates their interaction with leukocytes. (30, 90) For patients with complex and/or multivessel coronary artery disease, CABG surgery is considered a standard treatment with proven long-term safety and efficacy,(91-94) however, with SVG disease being a frequently observed concern. As several animal and clinical phase I and II studies supported P-selectin as a potential target in SVG disease, (34, 36, 95) we raised the hypothesis that the P-selectin antagonist inclacumab may prove efficient in reducing SVG disease after CABG surgery. The aim of this prospective, randomized, multicentre, double-blind, placebo-controlled trial was therefore to assess the effects of inclacumab, a monoclonal antibody directed against P-selectin, on SVG disease. A total of 384 patients undergoing elective or urgent CABG surgery were enrolled at 38 centers located in Canada and the United States, and randomized in a 1:1 ratio to receive inclacumab (20 mg/kg) or placebo at 4-weeks intervals during a treatment period of 32 weeks. The primary efficacy measure was the proportion of patients with diameter stenosis > 50% (including total occlusion) of at least 1 SVG on invasive angiography at 1 year as assessed by quantitative coronary angiography. This study demonstrated that inclacumab exerted no significant effect on the primary efficacy measure (26.4% versus 22.3% of patients in the placebo and inclacumab groups, adjusted odds ratio [OR] 0.80, 95% confidence interval [CI] 0.47-1.38, p=0.43).

A post hoc analysis revealed that inclacumab tended to reduce the primary efficacy measure in patients with higher as compared to those with lower baseline P-selectin levels (12.8% versus 27.8%, adjusted OR 0.37, 95% CI 0.12-1.15, p=0.085), findings which are interesting and need to be evaluated in future studies.

Hier findet sich folgende Arbeit: http://dx.doi.org/10.1016/j.jacc.2015.10.071, die aus urheberrechtlichen Gründen aus der elektronischen Version der Habilitationsschrift entfernt wurde.

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6. **Clinical criteria replenish high-sensitive troponin and inflammatory markers in the stratification of patients with suspected acute coronary syndrome.** Stähli BE, Yonekawa K, Altwegg LA, Wyss C, Hof D, Fischbacher P, Brauchlin A, Schulthess G, Krayenbühl PA, von Eckardstein A, Hersberger M, Neidhart M, Gay S, Novopashenny I, Wolters R, Frank M, Wischnewsky MB, Lüscher TF, Maier W. Plos One. 2014;9(6):e98626.

In patients presenting with symptoms suggestive of acute coronary syndromes, identification of those at increased risk and need for early coronary angiography with subsequent coronary revascularization if needed is paramount. High-sensitive cardiac biomarkers play a key role in the diagnosis of myocardial infarction, and markers of inflammation may complement patient assessment. The aim of this observational single center study was to establish a risk prediction tool for patients presenting with signs and symptoms of acute coronary syndromes. A total of 538 patients were screened, and 377 patients included in the study. On admission, the Thrombolysis in Myocardial Infarction (TIMI) and Global Registry of Acute Coronary Events (GRACE) risk scores were calculated for each patient, and a panel of 15 laboratory biomarkers was measured. The primary endpoint (cardiac event) was a composite of coronary revascularization, subsequent myocardial infarction, and cardiovascular death at 30 days. Coronary angiography and subsequent coronary revascularization were performed in 44% and 33% of patients, respectively. This study demonstrated that in patients presenting without ST-segment elevations, the performance of single biomarkers such as hs-TnT and myeloperoxidase in cardiac event prediction depended on the clinical pretest probability, with a better performance of hs-TnT in patients with low, and of myeloperoxidase in those with high clinical risk scores. Further, best prediction of cardiac events was achieved by combining clinical risk

scores with hs-TnT. These observations underline the importance of clinical parameters in the risk stratification of patients presenting with suspected acute coronary syndromes to the emergency department.

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DISCUSSION

The burden of coronary heart disease

Cardiovascular diseases are the most frequent cause of morbidity and mortality worldwide, with coronary heart disease accounting for the majority of them.(96) The total coronary heart disease prevalence in the United States is expected to be about 6% in adults over 20 years of age, meaning that an estimated 15.5 million Americans over 20 years of age are suffering from coronary heart disease,(1) and lifetime risk of fatal coronary heart disease or nonfatal myocardial infarction is considered to increase from 3.6% and below 1% for men and women with an optimal risk factor profile to 37.5% and 18.3% in those with 2 or more major cardiovascular risk factors.(97) Albeit a significant reduction of coronary heart disease mortality was achieved over the last decades given the better risk factor management and the improvements in pharmacological and interventional treatment, coronary heart disease is considered to account for every 7th death in the United States, and yet about a third of patients experiencing a coronary event will die of it in the same year.(1, 98). Similar rates have been reported in European countries with cardiovascular diseases causing about 4 million deaths each year, and coronary heart disease accounting for about half of them.(99) In near future, the burden of coronary heart disease is expected to rise further with the ageing of the population and the growing rates of obesity and diabetes, and an almost 20% increase in disease prevalence is projected by 2030.(1) The high prevalence of coronary heart disease and the associated morbidity and mortality, along with the related socioeconomic consequences, highlight the importance of both basic and clinical research efforts in this field to further enhance our understanding of pathophysiological aspects and to provide the basis for novel treatment approaches.

Hence, the aims of this habilitation thesis were 1) to elucidate the endothelial regulation of vascular tone under inflammatory conditions, 2) to investigate the

endothelial regulation of coagulation and develop novel therapeutic strategies, and 3) to improve risk stratification in patients with suspected acute coronary syndromes investigating the predictive role of inflammatory biomarkers.

Endothelial regulation of vascular tone

As highlighted above, the endothelium plays a key role in the regulation of vascular tone and maintains the balance between vasodilation and vasoconstriction. Nitric oxide is a key endothelial factor mediating endothelium-dependent vasodilation of the adjacent vascular smooth muscle cells.(2, 3) A variety of mediators are involved in the regulation of vascular tone under inflammatory conditions, and different responses to vasoactive substances among different vascular beds have previously been reported.(80, 100, 101) In this habilitation thesis, effects of histamine, known to be abundant in diseased coronary arteries,(102) and of PARP-1, orchestrating cellular responses to oxidative stress,(82, 83) on vascular function were further elucidated. Therefore, vessel segments of both human bypass conduits and mice aorta were collected and mounted in organ chambers for isometric tension recording. We demonstrated that histamine at lower concentrations induced relaxations in the internal mammary artery and to a lesser extent in the saphenous vein, but not in the radial artery due to minimal histamine H2 receptor expression in this vessel.(9) Histamine at higher concentrations, however, elicited contractions in all three vessels, mediated via the histamine H1 receptor. Similar responses to histamine in the saphenous vein with a relaxation at lower and a contraction at higher concentration have previously been reported.(101) As endothelium-dependent relaxations to acetylcholine were similar in the internal mammary and the radial artery, along with a comparable expression of the endothelial NO synthase in the two vessels, differences in the activation pattern of the NO pathway seem to explain the lack of histamine-induced relaxation in the radial

artery rather than the functionality of the NO pathway per se. The observation that histamine-induced relaxations in the internal mammary artery and the saphenous vein were blunted by removal of the endothelium and by the inhibitor of NO formation L-NAME confirms that these responses are mediated by NO derived from the endothelium. Cimetidine blocked relaxations to a similar extent as L-NAME both in the internal mammary artery and the saphenous vein, findings which strongly support the interpretation that the release of NO is mediated via histamine H2 receptor activation. Consistent with these findings, histamine H2 receptor-mediated vasodilation of coronary arteries has previously been reported in a dog model,(81) and cimetidine-induced coronary artery vasospasm was observed in patients with Prinzmetal angina.(103) As indomethacin did not alter histamine-induced vessel responses in the internal mammary and the radial artery, prostaglandins do not seem to be involved in the vessel responses observed in these arteries. However, as indomethacin unmasked histamine-induced relaxations in the saphenous vein, vasoconstrictive prostaglandins seem to counteract NO-mediated relaxations in these vessels. Taken together, the lack of histamine-induced NO release may represent a possible mechanism for radial artery vasospasm, and may be involved in the pathogenesis of bypass graft disease in this conduit as well. From a clinical perspective, as platelets are a main source of histamine,(104) consequent inhibition of platelet aggregation may be particularly important to reduce vasospastic complications in radial artery bypass grafts. Further, these findings add to the evidence that a heterogeneous distribution of receptors in the vascular bed accounts for different vessel responses to the same mediator.

Oxidative stress is a hallmark of endothelial dysfunction and atherosclerotic changes within the vessel wall. Given the importance of the nuclear enzyme PARP-1 in oxidative stress responses, we investigated the effects of genetic deletion of PARP-1 on endothelial function under conditions of oxidative stress. Poly(adenosine

diphosphate [ADP]-ribose) polymerase-1, by transferring ADP-ribose units to nuclear acceptor proteins, is importantly involved in deoxyribonucleic acid (DNA) repair mechanisms, thereby maintaining genomic stability,(105, 106) and was shown to promote endothelial integrity by mediating anti-apoptotic effects of the vascular endothelial growth factor.(107) Besides these beneficial properties, detrimental effects of PARP-1 activation are well known, and were mostly linked to the intracellular depletion of NAD^+ and ATP pools and the enhanced expression of pro-inflammatory mediators. Activation of PARP-1 has been shown to mediate tissue damage in animal models of diabetes and atherosclerosis,(108-110) and pharmacological PARP inhibition and genetic deletion of PARP-1 diminished endothelial adhesion molecule expression, reduced atherosclerotic plaque formation, and promoted plaque stability in mice models of atherosclerosis.(109) Our study showed that PARP-1 protects from oxidative stress induced endothelial dysfunction by inhibiting the production of cyclooxygenase-derived vasoconstrictor prostanoids.(111) These findings are in line with previous studies suggesting an increased production of vasoconstrictor prostanoids in response to oxidative stress in different animal models of vascular disease and diabetes.(112, 113) Contrary to our findings, beneficial effects of pharmacological PARP inhibition on endothelial function were observed in animal models of atherosclerosis and hypertension,(114-117) although effects may depend on the model used.(114) We can only speculate about the reasons for these diverse observations, however, the nature of the stimulus and the level of PARP-1 activation per se may play an important role in mediating beneficial or detrimental effects of PARP-1 activity.(118) Further, results obtained with pharmacological PARP inhibition may not be comparable to those observed in knockout mice, and different pharmacological PARP inhibitors may exert diverse effects or pleiotropic actions.

Future studies are needed to elucidate potential clinical implications of various PARP inhibitors in cardiovascular disease.

Two key players in coagulation and inflammation: tissue factor and P-selectin

The endothelium not only mediates vascular tone, but is also crucially involved in the regulation of coagulation. The research discussed in this habilitation thesis focuses on tissue factor and the adhesion molecule P-selectin, and was mainly fuelled by the ongoing search for improved stent designs and novel anti-thrombotic and anti-inflammatory treatment strategies in patients with acute coronary syndromes. Drug-eluting stents are covered with anti-proliferative agents to inhibit vascular smooth muscle cell proliferation, and thereby restenosis. Substances combining both anti-proliferative and anti-thrombotic properties therefore represent interesting candidate agents for the application on drug-eluting stents, and tissue factor as the main initiator of coagulation may be an interesting target.(14) This approach was further supported by the observation of our group that paclitaxel used on first-generation drug-eluting stents significantly increased endothelial tissue factor protein expression and surface activity via stabilization of microtubules and selective activation of the c-Jun terminal NH_2 kinase.(54) Therefore, we tested the effect of different agents on endothelial tissue factor expression. Ouabain is a cardiac glycoside which exerts its action via inhibition of the Na^+/K^+-ATPase, a protein located in the cellular membrane and regulating the active transport of sodium and potassium ions.(119) Previous studies have suggested that ouabain decreases vascular smooth muscle cell proliferation at higher concentrations.(85) We demonstrated that ouabain significantly reduced TNF-α-induced endothelial tissue factor expression, and that this effect was most likely mediated at the post-transcriptional level as neither TNF-α-induced MAP kinase activation nor IκB-α degradation were affected, and tissue factor protein degradation

remained unaltered.(120) The anti-proliferative and anti-thrombotic properties of ouabain may render this compound an interesting candidate agent for the application on drug-eluting stents. Further, these findings provide novel insights into the post-transcriptional regulation of tissue factor expression which has only rarely been reported before.(55) We then investigated the effect of the natural compound CAPE, a propolis component from honeybee hives, on endothelial tissue factor expression. Indeed, CAPE inhibited TNF-α-induced tissue factor expression, and similarly to ouabain, effects are considered to be mediated at the post-transcriptional level as neither the MAP kinase activation pattern, nor promoter activity or mRNA expression were altered.(121) Differences between the effects of both ouabain and CAPE on tissue factor protein expression and surface activity may be due to the distribution of tissue factor in various cellular compartments, along with the presence of encrypted tissue factor.(14) As caffeine consumption has frequently been linked with an increased risk of cardiovascular events such as acute myocardial infarction and stroke,(122-124) we further assessed whether caffeine exerts any effect on tissue factor expression. Caffeine significantly enhanced TNF-α- and thrombin-induced endothelial tissue factor expression via inhibition of PI3K activity with an effect comparable to that of the PI3K inhibitor LY294002.(125) As concentrations used in the study were comparable to those reached in humans after regular coffee consumption,(126, 127) these findings strongly support pro-thrombotic properties of caffeine and further underline the importance of the PI3K pathway in cardiovascular disease.

The second therapeutic concept discussed in this habilitation thesis involves the modulation of inflammatory and coagulation pathways by targeting the adhesion molecule P-selectin. The SELECT-CABG study demonstrated that the anti-P-selectin antibody inclacumab did not reduce venous graft failure in patients undergoing CABG

surgery,(128) suggesting that the P-selectin pathway seems to play an overall less important role in the pathogenesis of SVG disease than previously postulated. However, the fact that patients with elevated baseline levels of soluble P-selectin had a numerically lower rate of diseased SVG when treated with inclacumab compared to placebo raised the hypothesis that the pre-existing level of activation of the P-selectin pathway may determine the response to inclacumab in terms of SVG disease prevention. Although the identical dosage of inclacumab was proven to be successful in the recent SELECT-ACS trial enrolling patients with non-ST-segment elevation myocardial infarction undergoing PCI,(36) it cannot be excluded that treatment regimens involving pre-operative drug administration or longer treatment durations may have favorably affected the outcome measures.

Inflammatory biomarkers and their potential to improve risk prediction in coronary artery disease

As highlighted above, timely diagnosis and early risk stratification of patients presenting with signs and symptoms suggestive of acute coronary syndromes are important. However, the identification of patients at increased risk and need for early coronary revascularization may be challenging, particularly in patients presenting without ST-segment elevations. Different risk scoring systems have been established for the prediction of ischemic events and cardiovascular death such as the TIMI risk score which incorporates the variables age, presence of ≥3 cardiovascular risk factors, known coronary artery disease, episodes of angina, and the use of antiplatelet agents, along with positive cardiac biomarkers and ECG changes.(129) Although the implementation of hs-cTn assays in clinical practice has improved the diagnosis of myocardial infarction,(70-72) an increasing number of chest pain patients now presents with slight increases in cardiac troponin levels without finally being diagnosed

with acute coronary syndromes.(130, 131) Given the key role of inflammation in the pathogenesis of atherosclerosis, plaque rupture, and associated thrombotic complications, much research interest has been focused on markers of inflammation to further improve the assessment of patients presenting with suspected acute coronary syndromes. Inflammatory markers such as C-reactive protein (CRP) have been shown to predict the risk of cardiovascular events in both asymptomatic individuals and patients with established coronary artery disease,(132-134) and were linked to coronary plaque burden and atherosclerosis progression in patients undergoing coronary angiography.(135-137) Therefore, we tested different candidate biomarkers for the prediction of cardiac events (defined as need for coronary revascularization, consecutive myocardial infarction, and cardiovascular death at 30 days) in patients with suspected acute coronary syndromes.(138) The MyRiAd study demonstrated that clinical assessment by the TIMI risk score and hs-cTnT levels best predicted cardiac events, and that the predictive value of inflammatory biomarkers, particularly hs-cTnT and myeloperoxidase, depended on the clinical pretest probability as assessed by the TIMI risk score. The predictive value of inflammatory biomarkers in this study, however, was rather poor. Although H-FABP, a cytoplasmic protein released in response to myocardial injury, has previously been identified as an early marker of myocardial infarction and an independent predictor of major adverse cardiovascular events in patients with acute coronary syndromes,(139-141) consistent with other studies, this marker did not improve diagnostic accuracy beyond sensitive troponin assays.(142-144) Similarly, MRP 8/14, reflecting monocyte and granulocyte activation and known to be highly abundant in coronary thrombi,(74) did not significantly improve risk prediction. These findings may be rather disappointing as MRP 8/14 was shown to be elevated in patients with acute coronary syndromes,(74) and was linked with cardiovascular events both in healthy subjects and acute coronary

syndrome patients.(145, 146) Other biomarkers of plaque instability including the metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) and the leukocyte-derived enzyme myeloperoxidase did not improve diagnostic accuracy as compared with cardiac troponin assays, although they have been associated with an increased risk of adverse events in coronary artery disease and chest pain patients.(147-149) The observation that the predictive value of single biomarkers varied among different risk categories supports the integration of clinical variables and biomarker information in more complex risk prediction models. Indeed, similar algorithms incorporating both the TIMI risk score and cardiac troponin levels have been investigated in other studies enrolling patients with suspected acute coronary syndromes, and were found to accurately identify individuals at low risk of adverse events.(150-153) Other diagnostic concepts for an improved patient assessment involve multimarker strategies which may be superior to stand-alone testing of individual biomarkers. However, the best combination of candidate markers and their clinical role in the era of hs-cTn assays remains unclear.

Besides patients with suspected acute coronary syndromes, various candidate biomarkers have also been tested in other high-risk patient subsets. We have shown that carbamylated low-density lipoprotein (LDL) cholesterol not only elicited endothelial dysfunction via lectin-like-oxidized LDL receptor-1 activation, but also independently predicted adverse cardiovascular events and all-cause mortality in patients with chronic kidney disease.(154) Further, we demonstrated that emerging biomarkers such as midregional proadrenomedullin, neopterin, and tryptophan may bear the potential to improve risk prediction in aortic stenosis patients.(155-158) In coronary artery disease, imaging biomarkers may complement patient assessment beyond circulating biomarkers. Given the inability of conventional angiography and most intravascular imaging modalities to provide information about atherosclerotic plaque

composition and activity, near infrared fluorescence imaging and different molecular probes have been proposed for advanced intravascular plaque imaging,(159-162) and an novel bimodal intravascular ultrasound/near infrared fluorescence imaging system has recently been validated by our group in an animal model of atherosclerosis.(163)

Taken together, novel risk prediction algorithms combining different modalities such as clinical parameters, inflammatory biomarkers, and selected plaque imaging technologies, may further improve risk stratification in coronary artery disease patients in near future.

SUMMARY

In conclusion, aspects of endothelial dysfunction under inflammatory conditions with focus on vascular tone and coagulation were discussed in this habilitation thesis, which may provide the ground for future basic and clinical research. Further, novel therapeutic concepts acting at the interplay between inflammation and coagulation were investigated. In particular, the lack of histamine-induced NO production was identified as possible mechanism of vasospasm of the radial artery, and beneficial effects of the nuclear enzyme PARP-1 on endothelial function under conditions of oxidative stress were identified. In addition, anti-thrombotic effects of several compounds including cardiac glycosides and CAPE were demonstrated, and the role of a therapeutic strategy targeting P-selectin in SVG failure assessed with trends towards beneficial inclacumab effects observed in patients with high P-selectin levels. Further, we demonstrated that the performance of inflammatory biomarkers in predicting cardiac events depended on the clinical pretest probability assessed by the TIMI risk score. Taken together, these findings extend our knowledge about inflammatory alterations in cardiovascular disease both at the molecular level and from

a clinical perspective, and may influence future study designs aimed to further improve patient outcomes.

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