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Role of Tissue Factor in Thrombosis. Coagulation-Inflammation-Thrombosis circuit

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1. ABSTRACT

Tissue factor (TF) plays a role in thrombogenesis. TF initiates blood coagulation resulting in the generation of protease coagulant mediators (FVIIa, FXa, and FIIa) and fibrin production. TF hypercoagulablility directly contributes to thrombus formation resulting from the major events of fibrin deposition and FIIa-induced platelet activation/aggregation. In addition, blood coagulation indirectly promotes thrombogenicity via the coagulation-inflammation cycle in which TF plays a diverging and converging role. As the consequence of coagulation-dependent inflammation in which protease-activated receptor (PAR) mediates the coagulant signaling to elicit cytokines, selectins, and growth factors, such inflammation facilitates thrombosis by platelet aggregation and leukocyte recruitment. As TF hypercoagulability concerned, anti-thrombotic strategies involve the prevention by anticoagulation and PAR antagonism. Anticoagulants block the direct and indirect thrombotic contributions, while PAR antagonists arrest coagulation-dependent inflammation. With respect to both thrombosis and inflammation being cardiovascular risk factors, such strategies offer diverse benefits to cardioprotection

2. INTRODUCTION

Tissue factor (TF) initiates the extrinsic blood coagulation that is recognized as an integral player in the revised theory of coagulation (1). TF, an integral membrane glycoprotein (Mr. Wt. 43 kDa), is a known receptor (CD142) for clotting Factor VII (FVII) and its active form (FVIIa) serine protease (2). The extrinsic blood coagulation proceeds as an extracellular signaling cascade on phospholipids (PL)-containing membrane/surface in the presence of Ca^{+2} (3). As the consequence of TF exposure to FVII or FVIIa, an array of the activation of clotting factor zymogens (FX and prothrombin) results in the downstream generation of FXa and thrombin (FIIa) to propagate clotting signaling for fibrin production. In addition, TF plays diverse functions in such as inflammation (4), angiogenesis (5,6), tumor metastasis (6), embryonic development (7), cell adhesion/migration (8), the mediation of FVIIa signaling of anti-apoptosis (9), vascular endothelial growth factor (VEGF) expression (10), and many others (4).

TF upregulation is defined as its enhanced availability for exposure to FVII/FVIIa (11), which is responsible for hypercoagulability. TF expression is

susceptible to upregulation by shear stress (12), or diverse inflammatory events and cell activation in response to bacterial endotoxin, *Chlamydia* pneumoniae, cytokines, hypoxia, oxidized LDL, lipoprotein (a), homocysteine, phorbol esters, and many others (11). The activation of intracellular signaling kinases (e. g., protein kinase C, mitogen-activated protein kinase, protein tyrosine kinase) and transcription factors (e. g., nuclear factor-kappa B, activator protein-1, early growth response-1) mediates TF expression (11). For instance, TF hypercoagulability often results from sepsis (13) and vascular injury (14). Elevated plasma circulating TF (15) in such as myocardial infraction (MI) or apoptotic conditions (16) is also responsible for hypercoagulability. In some cases of intracellular Ca^{+2} activation, TF function is drastically upregulated without the increased protein synthesis (17).

Hypercoagulable state, an increasing tendency of thrombosis (18), is widely associated with various conditions such as diabetes, cancer and its therapy, oral contraceptives, cardiopulmonary bypass, and many others, all of which show elevated TF expression/activity (3). This review focuses on the contribution of TF hypercoagulability to thrombosis, a risk factor for cardiovascular events.

3. ROLE OF TF-INITIATED BLOOD COAGULATION IN THROMBOSIS

Thrombosis is generally characterized by its major events of fibrin deposition and platelet aggregation. Increasing evidence demonstrates TF-initiated blood coagulation playing a role in thrombogenesis (19). There is elevated TF level in deep vein thrombosis (DVT) (20); interestingly enough, macrovascular thrombosis is driven by TF derived primarily from the blood vessel wall but not leukocytes (21). FXa mediates TF/FVIIa-dependent arterial (22) or platelet- (23, 24) thrombus formation (24). In fact, TF (25), FVIIa (26) or FIIa (27) is able to induce platelet activation/aggregation consistent with the observation that recombinant FVIIa (rFVIIa) is of thrombogenicity (28). Treatment with anti-TF Ab attenuates thrombus formation (29, 30) and leukocyte activation/proliferation (31), indicating the participation of TF in thrombogenesis. Several lines of evidence suggest the antithrombotic potential of TF pathway inhibitor (TFPI). Local TFPI overexpression in vascular smooth muscle cell attenuates $FeCl_3$-induced thrombosis (32), while heterozygous TFPI deficiency show promoted thrombosis (33). Antibody against TFPI detected in antiphospholipid syndrome is correlated to increased thrombotic tendency and FIIa formation (34). Moreover, anticoagulation readily diminishes thrombosis (see the later section on Strategies for Antithrombosis; Table 1), which is in agreement with the involvement of blood coagulation in thrombosis.

Taken together, these observations are supportive to a notion that TF-initiated blood coagulation contributes to thrombogenesis. Such contribution to “fibrin” as well as “cellular” thrombosis results from the direct consequence of blood coagulation and/or a coupling with coagulation-dependent inflammation (Figure 1).

Other involvement of blood coagulation in thrombogenesis includes, for instance, that Von Willebrand factor facilitates platelet aggregation via interaction with activated glycoproteins (GP)IIb-IIIa and GPIb. Its binding to FVIII contributes to von Willebrand factor stability and function in the generation of fibrin, which is beyond the scope of this overview.

4. DIRECT CONTRIBUTION TO THROMBOGENESIS

Thrombosis characterized by thrombus formation results from fibrin overproduction (35). As the consequence of enhanced blood coagulation, elevated FIIa generation per se presents thrombotic risk (36), largely involving anti-fibrinolytic action and platelet aggregation.

4.1. “Fibrin” thrombosis

Fibrin overproduction under the condition of TF hypercoagulability leads to “fibrin” thrombosis not to mention its participation in enhanced thrombus formation by stabilizing platelet plug. Upon initial binding to TF, FVII zymogen undergoes proteolytic activation. The clotting signal is propagated; the resulting FVIIa/TF binary complex (the extrinsic Xase) catalyzes the activation of FX zymogen to FXa. Prothrombin is consequently converted to FIIa by the active prothromboinase complex consisting of FXa and FVa. Thereafter, soluble fibrinogen is cleaved at the N-terminal of alpha and beta chain by FIIa to release fibrinopeptide A and B, respectively. The exposed polymerizing sites are responsible for fibrin gel formation that is further stabilized and crosslinked by FXIIIa (1, 3). Thus, enhanced extrinsic blood coagulation results in fibrin overproduction.

4.2. Anti-fibrinolytic relevance

FIIa activates plasma carboxypeptidases recognized as thrombin-activatable fibrinolytic inhibitor (TAFI) that attenuates fibrinolysis (37) in favor of fibrin deposition/accumulation. Clinical studies showed that plasma TAFI level is correlated positively to venous/deep vein thrombosis (38), disseminated intravascular coagulation (39), the acute phase of ischemic stroke (40), and cardiovascular risks (41). TAFI inhibits various forms of plasminogen activator (PA)-mediated fibrinolysis (42). The inhibition is partially reversed by the addition of plasminogen, implying that TAFI modifies plasminogen more than PA binding to fibrin (43). TAFI cleaves fibrin C-terminal lysine residues that act as the binding sites for both plasminogen and PA, thereby diminishing plasminogen activation for plasmin formation (44). The released lysine forms Lys-plasminogen to undergo feedback inhibition on plasmin (44). Nesheim and his associates (45) have recently proposed a novel mechanism that TAFI reduces the ability of fibrin degradation products to protect plasmin from antiplasmin. Accordingly, the released lysine residues by TAFI action also suppress such protection (46), sustaining delayed fibrinolysis. In addition, the ability of FIIa to induce plasminogen activator inhibitor-1 (PAI-1) expression via a PKC-dependent mechanism (47) could further favor antifibrinolytic process and fibrin accumulation. Furthermore, FIIa activates FXIII, and FXIIIa facilitates the stabilization and crosslinking of fibrin clots.

Table 1. Anti-thrombotic effects of anticoagulants

Agent	Model	Inhibitory Effect	Application	Reference
TF downregulation				
anti-TF Ab	rabbits	thrombus formation	DVT; arterial/venous	29, 30
TF antisense	rats	leukocyte adhesion	ischemic reperfusion injury	114
TF/FVIIa or FVIIa inhibition				
FFR-rFVIIa	A-A shunt rats	occlusion time	thrombosis	115
FFR-rFVIIa	PCI patient blood	fibrin depoisition	thrombosis	116
DEGR-rFVIIa	*in vitro* whole blood	thrombus formation	arterial/venous	117
FVIIai	*in vitro* whole blood	platelet thrombus deposition	thrombosis	118
sTF	guinea pigs; rabbits	thrombus formation	arterial thrombosis	119
PN7051	*in vitro* whole blood	fibrin/platelet deposition & adhesion		120
PHA-798	primates	thrombus formation	DVT	121
FXa inhibition				
LMWH				
Fondaparinux	---------- clinical trials ----------		DVT; VTE	122
Enoxaparin	---------- clinical trials ----------		DVT; VTE; arterial/venous	123
Bemiparin	---------- clinical trials ----------		DVT; VTE	124
Tinzaparin	---------- clinical trials ----------		DVT; pulmonary embolism	125
Fraxiparin	---------- clinical trials ----------		venous thrombosis	126
Reviparin	----------COLUMBUS; CORTES----------		DVT; pulmonary embolism	127
Dalteparin	---------- clinical trials ----------		DVT; VTE	128
SamOrg123781A	pigs *ex vivo*	platelet adhesion & thrombus	arterial thrombosis	129
Direct inhibitor				
ATS; TAP	angioplasty rabbits	restenosis	thrombosis	130
TAP	*In vitro*	thrombus formation	arterial thrombosis	131
DX9065a	rabbits; canines	platelet aggregation	venous thrombosis	132,133
AAPPA	baboons	platelet & fibrin deposition	venous thrombosis	134
ZK-807834	vascular injury rabbits	thrombus formation	venous thrombosis	135
ZK-807834	electrically injury canines	thrombus formation	venous thrombosis	136
SF 303; SF 549	A-V shunt rabbits	thrombus formation	thrombosis	137
TM-75466	mice	thrombus formation	thromboembolism	138
FXV 673	canines	thrombus formation; occlusion	arterial thrombosis	139
DPC 423	electrically injury rabbits	*ex vivo* platelet aggregation	arterial thrombosis	140
Isoxazo-line/le	A-V shunt	*ex vivo* platelet aggregation	thrombosis	141
PRP 120844	rabbits; rats	thrombus formation	arterial/venous thrombosis	142
FIIa inhibition				
Heparins	---------- clinical trials ----------		DVT; VTE	143
Direct inhibitor				
hirudins	---------- clinical trials ----------		DVT;VTE; arterial thrombosis	144
ximelagatran	---------- clinical trials ----------		DVT; VTE	145
argatroban	---------- clinical trials ----------		DVT; VTE	146
Org 42675	rats/rabbits	occlusion	arterial thrombosis	147
Natural Anticoagulants				
TFPI				
rTFPI	*in vitro* whole blood	fibrin deposition	vascular thrombosis	148
TFPI 1-161	*in vitro* whole blood	fibrin deposition	venous thrombosis	149
APC				
LY203638	canines	thrombus formation	arterial thrombosis	155
CTC-111	mice	thrombus formation	venous thrombosis	156
FLIN-Q3	A-V shunt guinea pigs	thrombus formation	thrombosis	157
hAPC	rats	arterial occlusion	arterial thrombosis	158
rhAPC	baboons	thrombus formation	arterial thrombosis	159
bAPC	rats; rabbits	thrombus formation	microarterial thrombosis	160, 161
rbAPC	*in vitro*; *in vivo*	thrombus/platelet deposition	restenosis	162
AT III	ischemia/ reperfusion	leukocyte recruitment, neutrophil rolling & adhesion		163
Miscellaneous				
warfarin	---------- clinical trials ----------		DVT; VTE	164

A-V, arterio-venous; i. v., intravenous; PCI, percutaneous coronary intervention; sTF, soluble TF mutant; rTFPI, recombinant TFPI; TAP, tick anticoagulant protein; ATS, antistasin, APC, activated protein C; hAPC, human APC, rhAPC, recombinant human APC; bAPC, bovine APC; rbAPC, rabbit APC; AT III, antithrombin III; AAPPA, amidinoaryl propanoic acid.

4.3. Platelet aggregation

It has long been established that FIIa activates platelets mainly through protease-activated receptor (PAR) and GP. PAR-1 is a primary receptor for FIIa by which platelets are activated to aggregate (48). Platelet aggregation constitutes thrombus formation involving cross-linking of adjacent platelets mediated by the interaction of activated GP IIb/IIIa with distinct amino acid

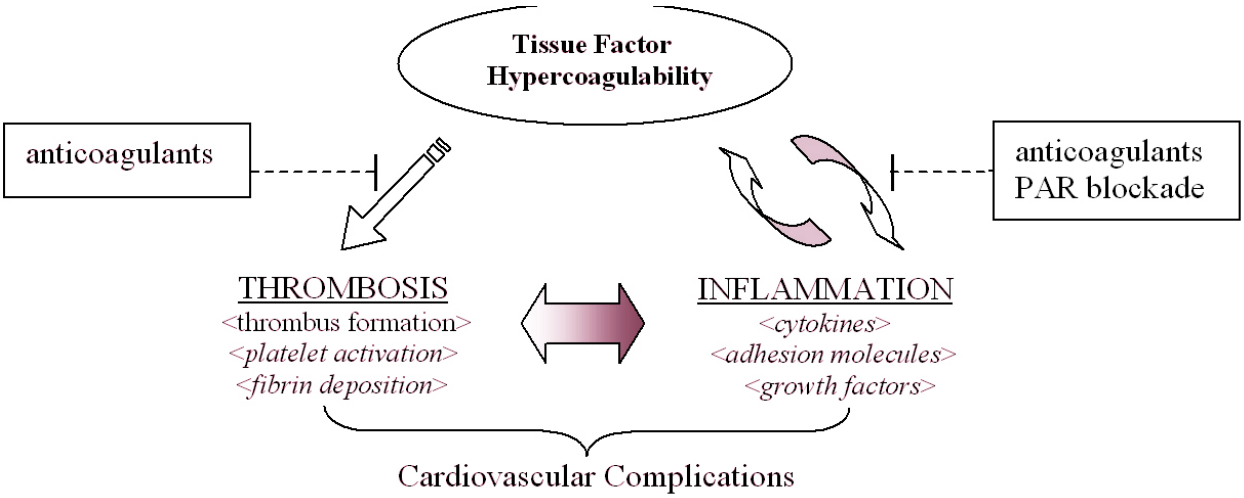

Figure 1. 'Coagulation-inflammation-thrombosis circuit': TF hypercoagulability leads to thrombosis and inflammation in relation to cardiovascular events. TF hypercoagulability directly (**arrow**) contributes to thrombosis by enhanced fibrin deposition and FIIa-induced platelet aggregation. Indirectly, the coagulation-inflammation cycle coupled with inflammation-dependent event (**two-way arrow**) participates in thrombogenesis. TF hypercoagulability drives a 'coagulation-inflammation-thrombosis circuit' in which inflammation (cytokines, adhesion molecules, and growth factors) and/or thrombosis (thrombus formation, platelet activation, and fibrin deposition) lead to cardiovascular complications. Anticoagulants inhibit (- - -) the direct thrombotic contribution, while anticoagulation and PAR antagonism interrupting (- - -) the coagulation-inflammation cycle (Figure 2) are antithrombotic.

sequences, LGGAKQAGDV and/or RGD, at each end of dimeric fibrinogen molecules (49). An alternative pathway describes that FIIa-induced platelet activation results from polymerizing fibrin, which involves the recognition sites in the cross-linking of polymerizing fibrin and surface integrins via GP Ib. In fact, GP Ib acts as a FIIa-binding site and promotes platelet activation by low FIIa concentrations (50). In addition, PAR-4-mediated FIIa action promotes leukocyte rolling and adhesion (51).

5. INDIRECT CONTRIBUTION MEDIATED BY COAGULATION-DEPENDENT INFLAMMATION

Serine proteases (FVIIa, FXa, FIIa) are not only coagulant but also proinflammatory mediators undergoing coagulation-dependent inflammation, which in turn leads to inflammation-dependent thrombotic consequence (4). Upon TF hypercoagulability, inflammatory consequence is enormous. Such 'endogenous' inflammation triggers thrombosis mainly by platelet aggregation and leukocyte recruitment. Hence, a 'circuit' (11) links among coagulation, inflammation and thrombosis, which is driven by TF hypercoagulability (Figure 1).

5.1. Coagulation-inflammation cycle

Hypercoagulability and inflammatory states derive each other, which is mediated by TF diverging and converging roles respectively for an inflammatory cause and consequence in a coagulation-inflammation cycle (Figure 2). Several lines of evidence have revealed *in vivo* **coagulation-dependent inflammation**. Anti-TF Ab prevents septic shock (52) and depresses macrophage proinflammatory functions in the expression of adhesion molecule CD18 (53), suggesting the proinflammatory role of TF. Administration with recombinant FVIIa enhances interleukin (IL)-6 and -8 productions in healthy human subjects (54). FXa/PL infusion increases the expression of IL-6 and C-reactive protein (CRP) in baboons (55). FIIa with fibrin(ogen) dependency induces the production of IL-6 and monocyte chemoattactant protein (MCP)-1 (56). IL-6 and CRP are proposed to be soluble benchmarks for the clinical diagnoses of inflammation.

Conversely, *in vivo* **inflammation-dependent coagulation** is evidenced by the intramuscular injection of IL-6 that results in FIIa generation in baboons (57). P-selectin leads to TF accumulation in the developing thrombi (58); its blockade by a recombinant soluble ligand-Ig (rPSGL-Ig) depresses TF mRNA expression (59). These observations are further in agreement with the positive correlation of TF procoagulant state with the high levels of soluble P-selectin in blood (60). Wakefield and his associates (61) have demonstrated that selectin-deficient mice are defective in fibrin production due to the lack of activation of the extrinsic pathway. In addition, CD40L (62) stronger than tumor necrosis factor-alpha (TNF) or IL-1 beta drastically induces TF activity proceeding with coagulation.

5.1.1. TF diverging role

TF initiates blood coagulation that in turn confers inflammatory state. The coagulant mediators (FVIIa, FXa, and FIIa) and fibrin production are responsible for triggering "endogenous" inflammation. Thus, TF plays a diverging role leading to **coagulation-dependent inflammation.** The inflammation involves eliciting TNF, IL-1, 6 & 8, MCP-1, intracellular adhesion molecule (ICAM), vascular cell adhesion molecule

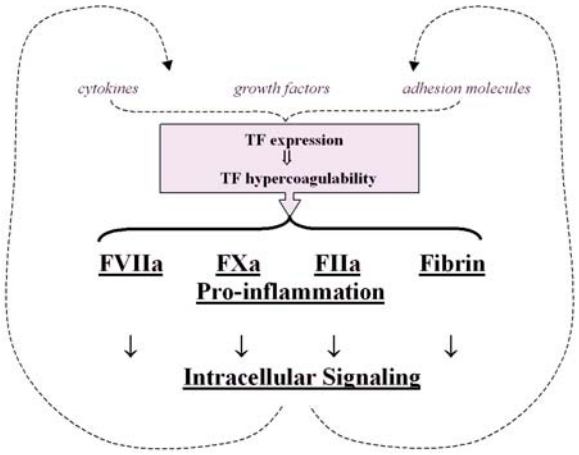

Figure 2. Unidirectional coagulation-inflammation cycle: TF hypercoagulability refueling the cycle results in enormous inflammation. TF expression couples coagulation to inflammation for sustaining the coagulation-inflammation cycle. TF diverging role (bold) undergoing coagulation-dependent inflammation (underline). As the consequence of TF hypercoagulability, elevated proinflammatory coagulant mediators (FVIIa, FXa, FIIa) and fibrin overproduction lead to upsurged elicitation of cytokines (e.g., TNF, ILs, etc.), adhesion molecules (e.g., MCP-1, ICAM/VCAM, selectins, etc.) and growth factors (e.g., VEGF, PDGF, etc.). On the other hand, TF converging role (dot line) presents its susceptibility to upregulation by such diverse inflammation (italics) for exhibiting hypercoagulation. Thus, TF converging and diverging role deriving each other manifest as hypercoagulable accompanying with inflammatory states. Namely, TF hypercoagulability continuously refuels the cycle in which coagulation and inflammation promote each other, despite how the cycle receives its initial momentum.

(VCAM), selectins, VEGF, platelet derived growth factor (PDGF), platelet activating factor, basic fibroblast growth factor, granule macrophage colony stimulating factor, *etc.* (11).

The signals of the coagulant mediators are largely transmitted by PAR, 7-transmembrane G-protein coupled receptors (63). The active protease including FVIIa, FXa, or FIIa undergoes a proteolytic cleavage of the extracellular domain of PAR, resulting in a new N terminus that in turn acts as a tethered ligand. Following the interaction with heterotrimeric G-proteins, the subsequent signaling kinases and/or intracellular Ca^{+2} signaling are activated. PAR-1 or -2-mediated FVIIa signaling activates Ca+2 and MAPK, and PAR-2-mediated signal promotes SMC migration. In TF-expressing cells, FVIIa is able to elicit VEGF expression (64). FXa signal is transduced by PAR-1, -2, or -3 to elicit IL-6 (6) & 8 (65), MCP-1 (66), VEGF (65), and PDGF (66) expression. FIIa activates different cell types (3); it's PAR-1 or -3-mediated signaling induces IL-6 (56, 65), IL-8 (65), MCP-1 (56, 65), ICAM (67), P-selectin (68), VEGF (69), and PDGF (70) expression. PAR-4-mediated FIIa action enhances leukocyte rolling and adhesion (51). In addition, fibrin and its fragments are also proinflammatory in eliciting IL-1 beta (71), IL-6 (72), IL-8 (72) and MCP-1 (73) through unknown non-PAR transduction.

The long-range effect extends to many cell types where PAR expressed ubiquitously facilitates smooth muscle cell proliferation (74) and activation of vascular endothelial cells (VEC) (75), platelets (76), or leukocytes (77), many of which are involved in thrombotic events.

5.1.2. TF converging role

On the other hand, TF is susceptible to upregulation; it converges various inflammatory signals including "exogenous" and "endogenous" ones. Such resulting TF upregulation exhibits hypercoagulable state. Thus, TF plays a converging role proceeding **inflammation-dependent coagulation**. It is noted that FVIIa (78), FXa (78, 79) and FIIa (80) promote TF expression, implying the existence of a feedback loop for completing the coagulation-inflammation cycle. The ability of PAR-2 agonists (e. g., trypsin (81), SLIGKV (81), and proteinase-3 (82)) to induce TF mRNA is in agreement with the upregulation by the proinflammatory coagulant mediators. In fact, TNF (83), ILs (84), MCP-1 (85), ICAM-1 (86), P-selectin (87), VEGF (88), PDGF (89) or many others (11) is able to substantially induce TF expression, which not only strengthens TF converging role but also supports such feedback upregulation on TF expression by the coagulant mediators (78-80). Moreover, the observations that anticoagulants (e.g., TFPI (90), FVIIai (91), DX9065a (79), ZK 807834 (92), low molecular weight heparins (LMWH) (93), heparin (94), hirudin (95), hirulog (96), antithrombin III (AT III) (97)) diminish TF expression are consistent with the occurrence of such an operative cycle.

In summary, TF drives the extracellular and intracellular signaling for coagulation and inflammation, respectively (Figure 2). The diverging and converging roles warrant TF hypercoaguability constantly refueling the cycle, resulting in enormous inflammation.

5.2. Inflammation triggering thrombosis

Inflammation-triggered thrombosis represents an example of the neighboring effect of the cycle on globally inflammatory impact. The clinical association of inflammation with thrombosis has been demonstrated (98). Several lines of evidence reveal *in vivo* **inflammation-dependent thrombogenesis**. IL-8 enhances fibrosis in rats (99). Antibodies to cytokines and adhesion molecules attenuate venous thrombosis (100). An earlier study has shown that P-selectin causes leukocyte accumulation to facilitate fibrin deposition (101) complementing thrombotic episodes. An antibody to P-selectin (LYP20) blocks leukocyte adhesion to EC and platelets (102) and modifies thrombosis (103). P-selectin inhibition decreases vein wall fibrosis (104). The observations that PAR antagonism is able to attenuate platelet activation/aggregation (see the later section on Strategies for Antithrombosis; Table 2) are in line with such indirect thrombotic contribution involving the coagulation-inflammation cycle as the consequence of TF hypercoagulability (Figure 1). At the cellular level, the

Table 2. Anti-thrombotic effect of PAR antagonism

Agent	Model/ Induction	Delivery	Inhibitory Effect	Reference
peptide mimetics				
RWJ 58259	*IN VITRO*/FIIa		human platelet aggregation	167
RWJ 58259	Monkey/vascular injury	i.v. catheter	vascular occlusion	168
RWJ 58259	Monkey/vascular injury	i.v. catheter	*ex vivo* platelet aggregation	168
RWJ 58259	Monkey/vascular injury	i.v. catheter	thrombus platelet deposition	168
RWJ 58259	rat/balloon angioplasty	perivascular	*in vivo* arterial stenosis	169
RWJ 58259	rat/balloon angioplasty	perivascular	neointimal thickness; restenosis	169
RWJ 58259	guinea pig A-V shunt	intrashunt	thrombus formation	169
RWJ 58259	guinea pig A-V shunt	i.v.	± thrombus formation	169
peptide antagonists				
SFLLR derivatives	*IN VITRO*/FIIa		human platelet aggregation	165, 166
non-peptide antagonists				
FR 171113	*IN VITRO*/FIIa		guinea pig platelet aggregation	170
FR 171113	guinea pig/FIIa	subcutaneous	*ex vivo* platelet aggregation	170
FR 171113	guinea pig/$FeCl_3$	subcutaneous	arterial thrombosis	170
FR 171113	guinea pig/ADP/collagen	subcutaneous	± *in vitro*/ *ex vivo* platelet aggregation	170
SCH 79797	*IN VITRO*/FIIa		human platelet aggregation	68
SCH 79797	*IN VITRO*/PAR-4/ADP/collagen		± human platelet aggregation	68
SCH 203099	*IN VITRO*/ FIIa		platelet surface P-selectin expression	68
YD-3	*IN VITRO*/GYPGKF		human platelet aggregation	171
YD-3	*IN VITRO*/GYPGKF		mouse platelet aggregation	171
YD-3	*IN VITRO*/fMLP/cathepsin G		human platelet aggregation	171
YD-3	*IN VITRO*/FIIa		mouse platelet aggregation	171
YD-3	*IN VITRO*/FIIa		± human platelet aggregation	171
other				
PAR-1 antibody	*ex vivo* monkey/FIIa	i.v. bolus	*ex vivo* platelet aggregation	173
PAR-1 antibody	rat/angioplasty	i.v.	SMC accumulation; neointimal thickness	172

i. v., intravenous; FIIa, thrombin; GYPGKF, a PAR-4 agonist; ±, no effect.

'endogenous' inflammation triggers platelet activation/adhesion and leukocyte recruitment in contribution to thrombus formation. VEGF potentiates FIIa-induced platelet aggregation (105). P/E/L-selectins, ICAM, and VCAM are responsible for leukocyte adhesion/ rolling/recruitment interacting with platelets and VEC to enhance thrombus formation (106). In contrast, *in vitro* exposure of whole blood to 'exogenous' TNF, IL-1, -6 and -8 fails to activate human platelets (107).

In addition, the general perception of inflammation-dependent thrombosis is supported by the observations that anti-inflammatory agents are of anti-thrombotic benefit. Non-steroid anti-inflammatory drugs readily block 'cellular' thrombosis. Cox-1 inhibitor such as low dose of aspirin suppresses platelet aggregation (108). Aspirin inhibits surface expression of GP IIb/IIIa, P-selectin, CD63, and CD107a receptor on human platelets (109). Similarly, Cox-2 inhibition downregulates VEC/leukocyte activation (110), while 5-lipoxygenase inhibition depresses leukocyte adhesion (111).

Conversely, **thrombosis-dependent inflammation** is also taking place by the ability of fibrin and its fragments to elicit IL-1beta, IL-6, IL-8 and MCP-1 expression (71-73). Further, platelet activation/aggregation participates in complement activation resulting in inflammatory responses. P-selectin as a C3b-binding protein sufficiently leads to C3a generation and C5b-C9 formation, which supports a novel mechanism of the local inflammation in vascular injury site.

6. STRATEGIES FOR ANTI-THROMBOSIS

Anti-thrombosis aims at the reduction of fibrin deposition and platelet aggregation. TF hypercoagulability confers thrombosis through the 'coagulation-inflammation-thrombosis circuit' involving the coagulation-inflammation cycle (Figure 1). Any interruption of such events is of prevention from thrombogenesis.

Anticoagulants target blood coagulation, shutting down the direct thrombotic contribution. As the result of anticoagulation, diminished formation of the proinflammatory coagulant signals (FVIIa, FXa, FIIa, and fibrin) reduces the indirect thrombotic inputs via inflammation-dependent thrombosis (Figure 1). In fact, anticoagulants readily suppress the elicitation of IL-1, 6 & 8, CRP, selectins, MCP-1, ICAM/VCAM, VEGF, PDGF, *etc*. (4).

In addition, PAR becomes a target for antiinflammation (4). PAR serves as a signal switcher bridging the extracellular signaling (blood coagulation) to the intracellular signaling (pro-inflammation) in the coagulation-inflammation cycle (Figure 2). Blocking the transmission of the proinflammatory coagulant signals, PAR antagonist holds the promises for anti-thrombotic applications. The role of PAR-1 in thrombogenesis is evident. PAR-1 deficiency (112) and knockout (113) show reduced thrombotic risk. PAR-1 is a primary receptor for FIIa by which platelets are activated to result in aggregation, manifesting platelet-dependent thrombosis.

6.1. Anticoagulation approach

Table 1 lists some typical antithrombotic examples resulting from anticoagulation by which blood clotting is blocked in different stages from the up- to down-stream events along the signaling cascade (3). Essentially, the initiation of the extrinsic pathway is inhibited by TF downregulation. The inhibition of TF/FVIIa binary complex and direct inhibition of FVIIa activity suppress FX activation and its downstream consequences. FXa inhibition by either AT III-dependent LMWH or direct FXa inhibitors blocks prothrombin activation (i.e., FIIa generation). FIIa inhibition is achieved by AT III-dependent heparins or direct FIIa inhibitors. Natural anticoagulants TFPI, activated protein C (APC), and AT III serve as a surveillance system to modulate blood coagulation. TFPI forms ternary complex with TF/FXa to inhibit FVIIa function. APC coupled with protein S inactivates FVa and FVIIIa to downregulate prothrombinase and the intrinsic tenase, respectively. ATIII mediates the diverse inhibitory actions of heparins and LMWHs.

The antithrombotic effects of LMWHs (anti-FXa), heparin (anti-FIIa) and direct FIIa inhibitors have been reviewed elsewhere based on clinical trials; the attentions of this section are paid to those experimental agents with antithrombotic potentials.

6.1.1. TF downregulation

Anti-TF antibody blocks TF function. An i.v. delivered antibody (AP-1) against rabbit TF inhibits intravascular thrombosis (29) and thrombus propagation without affecting bleeding time in rabbits (30). The antisense oligonucleotide blocking TF expression prevents leukocyte adhesion following renal ischemic reperfusion injury (114). One could also expect that inhibition of TF synthesis interrupts the cycle to prevent thrombosis. However, little is known about the antithrombotic relevance of targeting TF synthesis. The inconclusive issue is attributed to the fact that various inhibition of the intracellular signaling readily shows anti-inflammation apart from the downregulation of TF synthesis (4).

6.1.2. TF/FVIIa inhibition

Inactivated rFVIIa competes the native FVIIa affinity for TF, thereby inhibiting TF/FVIIa activity. i.v. Bolus of FFR-rFVIIa reduces thrombus and fibrin deposition in A-A shunt rat model (115). FFR-rFVIIa inhibits *ex vivo* fibrin deposition in patients undertaking percutaneous coronary intervention (PCI) (116). DEGR-rFVIIa prevents thrombus formation in whole blood (117). Similarly, an active-site blocked FVIIai attenuates fibrin/platelet deposition (118). By altering TF/FVIIa binding and inhibiting its activity, soluble TF mutant reduces arterial thrombosis in guinea pigs (119). A cyclic dodecapeptide (PN7051) derived from the second EGF-like domain of FVII interferes with TF/FVII/FX complex to attenuate fibrin deposition, platelet-fibrin adhesion and platelet-thrombus formation (120). PHA-798 diminishes thrombus formation in primates (121). It remains to be determined concerning the antithombotic application of nematode anticoagulant protein c2, a novel inhibitor for TF/FVIIa complex (3).

6.1.3. Anti-FXa

LMWHs including Fondaparinux (122), Enoxaparin (123), Bemiparin (124), Tinzaparin (125), Fraxiparin (126), Reviparin (127), and Dalteparin (128) exhibit clinical benefits for arterial and venous thrombosis. All LMWHs are able to markedly inhibit platelet aggregation in whole blood. Fondaparinux, a pentasaccharide, is widely reported to prevent venous thromboembolism (VTE) and treat DVT. SamOrg 123781A has recently been evaluated for its antithrombotic application with reduced platelet adhesion and thrombus formation in pigs (129).

Direct FXa inhibitors block the active sites. Recombinant antistasin (rATS) or tick anticoagulant peptide (rTAP) reduces restenosis in balloon angioplasty rabbits (130), and rTAP reduces TF/FVIIa-dependent thrombus formation *in vitro* (131). Non-peptide small molecules directly inhibit FXa activity. DX-9065a depresses platelet aggregation (132) and leukocyte adhesion to EC (133). Orally active amidinoaryl propanoic acid (AAPPA) reduces platelet deposition and fibrin accumulation in venous-type thrombus in baboons (134). ZK-807834 inhibits venous thrombosis in vascular injury rabbits (135) and electrolytic injury canines (136). SF 303 and 549 inhibit A-V shunt-induced thrombus formation in rabbits (137). Orally active YM-75466 inhibits thrombosis in mice (138). FXV673 inhibits thrombus formation in canines (139). Orally active pyrazole DPC423 attenuates electrically induced carotid artery thrombosis in rabbits (140). Isoxazolines and isoxazoles prevent A-V shunt-thrombosis (141), while RPR120844 reduces venous thrombosis in rabbits (142).

6.1.4. Anti-FIIa

The Hirsh group and many other investigators have shown a broad spectrum of the antithrombotic action of heparins in adults, pregnant women, and pediatric population (143). Direct FIIa inhibitors: hirudin derivatives (e.g. lepirudin, desirudin) and hirudin analogues (e.g. bivalirudin) are bivalent direct thrombin inhibitors binding to two distinct sites on thrombin: active (catalytic) and fibrinogen-binding site (exosite 1); these inhibitors (144) exhibit antagonism to DVT, VTE, arterial thrombosis in clinical studies. Ximelagatran (145), an active site inhibitor, shows various antithrombotic actions; so does argatroban attenuate DVT and VTE (146). Org 42675 is a direct anti-

FIIa agent with anti-FXa activity, seemingly being superior to argatroban and fondaparinux in animal models of thrombosis (147).

6.1.5. Natural anticoagulants

Consistent with the role of TFPI (32-34), treatment with rTFPI exhibits antithrombotic effect in a human *ex vivo* thrombotic model (148), and a truncated TFPI 1-161 reduces thrombus formation (149).

The role of APC in thrombosis has been demonstrated by the increased risk in APC resistance (150), deficiency (151) and low plasma level (152). In addition to its function in anticoagulation, APC exerts profibrinolytic effects by inactivation of PAI-1 (153) and TAFI (154), all of which synergistically diminishing the direct thrombotic inputs from blood coagulation cascade (Section 4). Moreover, effective APC anti-inflammatory actions (4) could also block the indirect contribution via inflammation-dependent thrombotic consequence. APC antithrombotic potential remains in the experimental stage of animal studies. A recombinant human APC (LY203638) inhibits arterial thrombosis in a canine model (155). A human APC product (CTC-111) reduces venous thrombosis in mice (156). FLIN-Q3 diminishes A-V shunt-induced thrombosis in guinea pigs (157). hAPC attenuates rat mesenteric occlusion (158), and rhAPC inhibits arterial thrombosis in baboons (159). Infusion of bovine APC suppresses thrombus formation in rats (160) and rabbit microarterial thrombosis (161). A rabbit APC-loaded stent reduces thrombus and platelet deposition *in vitro* and *in vivo* (162).

Little is known about the antithrombotic application of AT III despite some anti-inflammatory interests (4). A bolus infusion with ATIII attenuates FIIa-induced leukocyte rolling/adhesion/ recruitment in ischemia/reperfusion (163).

6.1.6. Other

Although warfarin generally blocking vitamin K-dependent zymogen activations in the coagulation cascade exhibits antithrombotic applications (164), its therapeutical use is limited by the drawback of fatal bleeding episodes.

6.2. PAR blockade

Table 2 lists the availability of PAR antagonists classified into three categories. Peptide mimetics such as RWJ® compounds are developed on the bases of PAR-1 agonist (SFLLRN) substituted with chemical modified non-proteogenic amino acids. Peptide antagonists include the chemical modified SFLLR substituted with *p*-fluoro (165) or *p*-guanido (166) -phenylalanine. Non-peptide PAR-1 antagonists such as FR® and SCH® compounds have been tested for their antithombotic potentials. Little is known about the antithrombotic relevance of PAR-2 antagonists. Thus far, there is no availability of PAR-3 antagonist.

6.2.1. Peptide mimetics

RWJ-58259 significantly reduces thrombus platelet deposition *in vitro* (167), monkeys (168), and rats (169) regardless of the induction by FIIa, balloon angioplasty or vascular injury.

6.2.2. Non-peptide antagonist

Subcutaneous FR 171113 preferentially diminishes FIIa-induced platelet aggregation without prolongation of bleeding time in guinea pigs (170). SCH 79797 and its N-methyl analog (SCH 203099) inhibit binding of a high-affinity FIIa receptor-activating peptide, diminishing *in vitro* platelet aggregation induced by FIIa (67, 68). YD-3, a PAR-4 antagonist, preferentially blocks GYPGKF (a PAR-4 agonist), fMLP, or cathepsin-mediated *in vitro* platelet aggregation (171), while it remains neutral on FIIa and PAR-1-induced action.

6.2.3. peptide antagonist

SFLLR derivatives inhibit human platelet aggregation *in vitro* (165, 166); their *in vivo* antithrombotic applications remain to be further elucidated.

6.2.4. Other

PAR-1 blocking antibody shows antithrombotic effects in rats (172) and monkeys (173), while PAR-1 antisense has no effect on platelet aggregation.

6.3. Other approaches interrupting the cycle

The general inflammatory blockade by antibody to CD14, LPS binding protein, Toll-like receptors, IL-6, MCP-1, or TNF could also be applicable to arresting TF upregulation and its consequence of inflammation-dependent thrombosis. Further work warrants determining the feasibility and antithrombotic applications.

7. REMARKS

TF hypercoagulability drives a 'coagulation-inflammation-thrombosis circuit' involving the coagulation-inflammation cycle in contribution to cardiovascular complications (Figure 1). TF hypercoagulability extends two arms conferring thrombosis and inflammation. Inflammation per se presents cardiovascular risks (174) not to mention its close link to thrombosis, a precursor to vascular dysfunction (175).

Anticoagulants and PAR antagonists respectively diminish the formation and the signal transmission of the coagulant mediators to interrupt the coagulation-inflammation cycle (Figure 2) and the 'coagulation-inflammation-thrombosis circuit' (Figure 1). Accordingly, anticoagulation and PAR blockade are of relevance to cardioprotection. Anticoagulants exhibit the management on cardiovascular disorders (176). rFVIIai benefits to acute coronary syndromes (ACS) (177). Enoxaparin is effective for treating unstable angina (175)/ MI (178)/ PCI (179)/ ischemic heart disease (180) and ACS in the SYNERGY randomized trial (181). Biralirudin is beneficial to not only PCI in the REPLACE trial (182), but also ACS (183), post MI and non-PCI unstable angina (184). In addition, anticoagulants draw attentions to arresting atrial fibrillation and preventing secondary stroke (e.g., enoxaparin (185) and ximelagatran (186) in relieving atrial fibrillation, biralirudin in acute ischemic stroke intervention (187), and ximelagatran in stroke prevention (186, 188)). However, anticoagulation often accompanies bleeding episode resulting from FIIa inhibition and warfarin, both of which

overkill blood coagulation and damage haemostatic balance. In general, anti-FXa is superior to anti-FIIa in view of less bleeding event. The development of TF/FVIIa inhibition is urged to meet such challenge.

PAR-1 (189) and -2 (190) recently become interesting targets in cardiovascular therapy. PAR blockade diminishes coagulation-dependent inflammation without affecting blood coagulation. Nor is bleeding reported. Further clinical trials are needed to determine if their anti-thombotic efficacies are advantageous over those of anticoagulants. It also remains challenging to evaluate the combined therapy of anticoagulants and PAR antagonists offering further cardioprotection.

8. REFERENCES

1. Broze G. J. Jr.: Tissue pathway inhibitor and revised theory of coagulation. *Annu Rev Med* 46, 103-112 (1995)
2. Edgington T. S., Dickinson C.D. & W. Ruf: The structural basis of function of the TF/VIIa complex in the cellular initiation of coagulation. *Thromb Haemost* 78, 401-405 (1997)
3. Chu A. J.: Biochemical strategies to anticoagulation: a comparative overview. *Curr Vasc Pharmacol* 2**,** 199-228 (2004)
4. Chu A. J.: Tissue factor mediates inflammation. *Arch Biochem Biophys* (2005) (in press)
5. Versteeg H. H., M. P. Peppelenbosch & C. A. Spek: Tissue factor signal transduction in angiogenesis. *Carcinogenesis* 24, 1009-1013 (2003)
6. Fernandez P. M. & F. R. Rickles: Tissue factor and angiogenesis in cancer. *Curr Opin Hematol* 9, 401-406 (2002)
7. Rosen E.D., J. C. Chan, E. Idusogie, F. Clotman, G. Vlasuk, T. Luther, L.R. Jalbert, S. Albrecht, L. Zhong, A. Lissens, L. Schoonjans, L. Moons, D. Collen, F. J. Castellino & P. Carmeliet: Mice lacking factor VII develop normally but suffer fatal perinatal bleeding. *Nature* 390, 290-294 (1997)
8. Ott I., E. G. Fischer, Y. Miyagi, B. M. Mueller & W. Ruf: A role for tissue factor in cell adhesion and migration mediated by interaction with actin-binding protein 280. *J Cell Biol* 140, 1241-1253 (1998)
9. Sorensen B. B., L. V. Rao, D. Tornehave, S. Gammeltoft & L. C. Petersen: Antiapoptotic effect of coagulation factor VIIa. *Blood* 102, 1708-1715 (2003)
10. Ollivier V., S. Bentolila, J. Chabbat, J. Hakim & D. de Prost: Tissue factor-dependent vascular endothelial growth factor production by human fibroblasts in response to activated factor VII. *Blood* 91, 2698-2703 (1998)
11. Chu A. J.: Tissue factor upregulation drives a thrombosis-inflammation circuit in relation to cardiovascular complications. *Cell Biochem Funct* Dec 22; [Epub ahead of print] (2004)
12. Lin M. C., F. Almus-Jacobs, H. H. Chen, G. C. Parry, N. Mackman, J. Y. Shyy & S. Chien: Shear stress induction of the tissue factor gene. *J Clin Invest* 99, 737-744 (1997)
13. Nimah M. & R. J. Brilli: Coagulation dysfunction in sepsis and multiple organ system failure. *Crit Care Clin* 19, 441-458 (2003)
14. Lorenzet R., E. Napoleone, A. Celi, G. Pellegrini & A. Di Santo: Cell-cell interaction and tissue factor expression. *Blood Coagul Fibrinolysis* 9 (S), S49-59 (1998)
15. Giesen P. L. & Y. Nemerson: Tissue factor on the loose. *Semin Thromb Hemost* 26, 379-384 (2000)
16. Tedgui A. & Z. Mallat: Apoptosis, a major determinant of atherothrombosis. *Arch Mal Coeur Vaiss* 96, 671-675 (2003)
17. Bach R. R. & C. F. Moldow: Mechanism of tissue factor activation on HL-60 cells. *Blood* 89, 3270-3276 (1997)
18. Thomas R. H. Hypercoagulability syndromes. *Arch Intern Med*. 161, 2433-2439 (2001)
19. Hathcock J.: Vascular biology-the role of tissue factor. *Semin Hematol* 41(S1), 30-34 (2004)
20. Smith A., J. W. Quarmby, M. Collins, S M. Lockhart & K. G. Burnand: Changes in the levels of soluble adhesion molecules and coagulation factors in patients with deep vein thrombosis. *Thromb Haemost* 82, 1593-1599 (1999)
21. Day S. M., J. L. Reeve, B. Pedersen, D. M. Farris, D. D. Myers, M. Im, T. W. Wakefield, N. Mackman N & W. P. Fay: Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood* 105,192-198 (2005)
22. Barstad R. M., M. J. Hamers, R. W. Stephens & K. S. Sakariassen: Retinoic acid reduces induction of monocyte tissue factor and tissue factor/factor VIIa-dependent arterial thrombus formation. *Blood* 86, 212-218 (1995)
23. Barstad R. M., M. J. Hamers, P. Kierulf, A. B. Westvik & K. S. Sakariassen: Procoagulant human monocytes mediate tissue factor/factor VIIa-dependent platelet-thrombus formation when exposed to flowing nonanticoagulated human blood. *Arterioscler Thromb Vasc Biol* 15, 11-16 (1995)
24. Speidel C. M., J. D. Thornton, Y. Y. Meng, P. R. Eisenberg, T. S. Edgington & D. R. Abendschein: Procoagulant activity on injured arteries and associated thrombi is mediated primarily by the complex of tissue factor and factor VIIa. *Coron Artery Dis* 7, 57-62 (1996)
25. Ahmad S., W. P. Jeske, Q. Ma, J. M. Walenga & J. Fareed: Inhibition of tissue factor-activated platelets by low-molecular-weight heparins and glycoprotein IIb/IIIa receptor antagonist. *Thromb Res* 102, 143-151 (2001)
26. Carr M. E. Jr., E. J. Martin, J. G. Kuhn & S. V. Seremetis: Effects of recombinant factor VIIa on platelet function and clot structure in blood with deficient prothrombin conversion. *Thromb Haemost* 89, 803-811 (2003)
27. Bouchard B. A., S. Butenas, K. G. Mann & P. B. Tracy: Interaction between platelets and the coagulation system. In Platelets (Ed: Michelson A.) Academic Press, San Diego, p 229-253 (2002)
28. Aledort L. M.: rFVIIa--its thrombogenicity. *Thromb Haemost* 84, 522-523 (2000)
29. Pawashe A. B., P. Golino, G. Ambrosio, F. Migliaccio, M. Ragni, I. Pascucci, M. Chiariello, R. Bach, A. Garen, W. K. Konigsberg: A monoclonal antibody against rabbit tissue factor inhibits thrombus formation in stenotic injured rabbit carotid arteries. *Circ Res* 74, 56-63 (1994)
30. Himber J., C. Wohlgensinger, S. Roux, L. A Damico, J. T. Fallon, D. Kirchhofer, Y. Nemerson & M. A. Riederer: Inhibition of tissue factor limits the growth of

venous thrombus in the rabbit. *J Thromb Haemost* 1, 889-895 (2003)
31. Annex B. H., M. G. Davies, G. J. Fulton, T. T. Huynh, K. M. Channon, M. D. Ezekowitz & P. O. Hagen: Local delivery of a tissue factor antibody reduces early leukocyte infiltration but fails to limit intimal hyperplasia in experimental vein grafts. *J Surg Res* 80, 164-170 (1998)
32. Pan S., L. S. Kleppe, T. A. Witt, C. S. Mueske & R. D. Simari: The effect of vascular smooth muscle cell-targeted expression of tissue factor pathway inhibitor in a murine model of arterial thrombosis. *Thromb Haemost* 92, 495-502 (2004)
33. Westrick R. J., P. F. Bodary, Z. Xu, Y. C. Shen, G. J. Broze & D. T. Eitzman: Deficiency of tissue factor pathway inhibitor promotes atherosclerosis and thrombosis in mice. *Circulation* 103, 3044-3046 (2001)
34. Adams M., L. Breckler, P. Stevens, J. Thom, R. Baker & R. Oostryck: Anti-tissue factor pathway inhibitor activity in subjects with antiphospholipid syndrome is associated with increased thrombin generation. *Haematologica* 89, 985-990 (2004)
35. Harker L. A.: Pathogenesis of thrombosis. In Hematology (Eds: Williams WJ, Beutler E, Erslev AJ, Lictman MA.) McGraw-Hill, New York, 1559-1568 (1990)
36. Harker L. A., S. R. Hanson & M. Runge: Thrombin hypothesis of thrombus generation and vascular lesion formation. *Am J Cardio* 75, 12B-17B (1995)
37. Bouma B. N. & J. C. Meijers: New insights into factors affecting clot stability: A role for thrombin activatable fibrinolysis inhibitor (TAFI; plasma procarboxypeptidase B, plasma procarboxypeptidase U, procarboxypeptidase R) *Semin Hematol* 41 (S1), 13-19 (2004)
38. van Tilburg N. H., F. R. Rosendaal & R. M. Bertina: Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis *Blood* 95, 2855-2859 (2000)
39. Watanabe R., H. Wada, Y. Watanabe, M. Sakakura, T. Nakasaki, Y. Mori, M. Nishikawa, E. C. Gabazza, T. Nobori & H. Shiku: Activity and antigen levels of thrombin-activatable fibrinolysis inhibitor in plasma of patients with disseminated intravascular coagulation. *Thromb Res* 104, 1-6 (2001)
40. Montaner J., M. Ribo, J. Monasterio, C. A. Molina & J. Alvarez-Sabin: Thrombin-activable fibrinolysis inhibitor levels in the acute phase of ischemic stroke. *Stroke* 34, 1038-1040 (2003)
41. Juhan-Vague I., J. F. Renucci, M. Grimaux, P. E. Morange, J. Gouvernet, Y. Gourmelin & M. C. Alessi: Thrombin-activatable fibrinolysis inhibitor antigen levels and cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 20, 2156-2161 (2000)
42. Guimaraes A. H. & D. C. Rijken: Thrombin activatable fibrinolysis inhibitor (TAFI) affects fibrinolysis in a plasminogen activator concentration-dependent manner. Study of seven plasminogen activators in an internal clot lysis model. *Thromb Haemost* 91, 473-479 (2004)
43. Mutch N. J., N. R. Moore, E. Wang & N. A. Booth: Thrombus lysis by uPA, scuPA and tPA is regulated by plasma TAFI. *J Thromb Haemost* 1, 2000-2007 (2003)
44. Wang W., M. B. Boffa, L. Bajzar, J. B. Walker & M. E. Nesheim: A study of the mechanism of inhibition of fibrinolysis by activated thrombin-activable fibrinolysis inhibitor. *J Biol Chem* 273, 27176-2781 (1998)
45. Schneider M., N. Brufatto, E. Neill & M. Nesheim: Activated thrombin-activatable fibrinolysis inhibitor reduces the ability of high molecular weight fibrin degradation products to protect plasmin from antiplasmin. *J Biol Chem* 279, 13340-13345 (2004)
46. Schneider M. & M. Nesheim: A study of the protection of plasmin from antiplasmin inhibition within an intact fibrin clot during the course of clot lysis. *J Biol Chem* 279, 13333-13339 (2004)
47. Mandl-Weber S., B. Haslinger & T. Sitter: Thrombin upregulates production of plasminogen activator inhibitor type 1 in human peritoneal mesothelial cells. *Perit Dial Int* 19, 319-324 (1999)
48. Tappaelli C., R. Metternich & N. S. Cook: Structure and function of thrombin receptors. *Trends Pharmcol Sci* 14, 426-429. (1993)
49. Soslau G., R. Class, D. A. Morgan, C. Foster, S. T. Lord, P. Marchese & Z. M. Ruggeri: Unique pathway of thrombin-induced platelet aggregation mediated by glycoprotein Ib. *J Biol Chem* 276, 21173-21183 (2001)
50. Adam F., M. C. Guillin & M. Jandrot-Perrus: Glycoprotein Ib-mediated platelet activation. A signalling pathway triggered by thrombin. *Eur J Biochem* 270, 2959-2970 (2003)
51. Vergnolle N., C. K. Derian, M. R. D'Andrea, M. Steinhoff & P. Andrade-Gordon: Characterization of thrombin-induced leukocyte rolling and adherence: a potential proinflammatory role for proteinase-activated receptor-4. *J Immunol* 169, 1467-1473 (2002)
52. Taylor F.B. Jr., A. Chang, W. Ruf, J. H. Morrissey, L. Hinshaw, R. Catlett, M. S. Lockhart & C. T. Esmon: Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. *Circ. Shock* 33, 127-134 (1991)
53. Cunningham M. A., P. Romas, P. Hutchinson, S. R. Holdsworth & P. G. Tipping: Tissue factor and factor VIIa receptor/ligand interactions induce proinflammatory effects in macrophages. *Blood* 94, 3413-3420 (1999)
54. De Jonge E., P. W. Friederich, G. P. Vlasuk, W. E. Rote, M. B. Vroom, M. Levi & T. van der Poll: Activation of coagulation by administration of recombinant factor VIIa elicits interleukin 6 (IL-6) and IL-8 release in healthy human subjects. *Clin Diagn Lab Immunol* 10, 495-497 (2003)
55. Kruithof E. K, D. Agay, J. C. Mestries, M. P. Gascon & A.Ythier: The effect of factor Xa/phospholipid infusion on the acute phase response in baboons. *Thromb Haemost* 77, 308-311 (1997)
56. Szaba F. M. & S. T. Smiley: Roles for thrombin and fibrin(ogen) in cytokine/chemokine production and macrophage adhesion *in vivo*. *Blood* 99, 1053-1059 (2002)
57. Kruithof E. K., J. C. Mestries, M. P. Gascon & A. Ythier: The coagulation and fibrinolytic responses of baboons after *in vivo* thrombin generation--effect of interleukin 6. *Thromb Haemost* 77, 905-910 (1997)
58. Falati S., Q. Liu, P. Gross, G. Merrill-Skoloff, J. Chou, E. Vandendries, A. Celi, K. Croce, B. C. Furie & B. Furie: Accumulation of tissue factor into developing thrombi *in vivo* is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin *J Exp Med* 197, 1585-1598 (2003)
59. Armstead V. E., A. G.Minchenko, R. Scalla & A. M.Lefer: Pulmonary tissue factor mRNA expression during murine traumatic shock: effect of P-selectin blockade. Shock 15, 323-326 (2001)

60. Andre P., D.Hartwell, I. Hrachovinova, S. Saffaripour & D. D. Wagner: Procoagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci USA* 97, 13835-13840 (2000)
61. Sullivan V. V., A. E. Hawley, D. M. Farris, B. S. Knipp, A. J. Varga, S. K. Wrobleski, P. Thanapron, M. J. Eagleton, D. D. Myers, Jr, J. B. Fowlkes & T. W. Wakefield: Decrease in fibrin content of venous thrombi in selectin-deficient mice. *J Surg Res* 109, 1-7 (2003)
62. Mach F., U. Schonbeck, J. Y. Bonnefoy, J. S. Pober & P. Libby: Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. *Circulation* 96, 396-399 (1997)
63. Mackie E. J., C. N. Pagel, R. Smith, M. R. de Niese, S. J. Song & R. N. Pike: Protease-activated receptors: a means of converting extracellular proteolysis into intracellular signals. *IUBMB Life* 53**,** 277-281 (2002)
64. Ollivier V., S. Bentolila, J. Chabbat, J. Hakim & D. de Prost: Tissue factor-dependent vascular endothelial growth factor production by human fibroblasts in response to activated factor VII. *Blood* 91, 2698-2703 (1998)
65. Bachli E. B., C. M. Pech, K. M. Johnson, D. J. Johnson, E. G. Tuddenham & J. H. McVey: Factor Xa and thrombin, but not factor VIIa, elicit specific cellular responses in dermal fibroblasts. *J Thromb Haemost* 9, 1935-1944 (2003)
66. Lan R. S., D. A.Knight, G. A. Stewart & P. J. Henry: Role of PGE(2) in protease-activated receptor-1, -2 and -4 mediated relaxation in the mouse isolated trachea. *Br J Pharmacol* 131, 689-694 2000)
67. Rahman A., A. L. True, K. N. Anwar, R. D. Ye, T. A. Voyno-Yasenetskaya & A. B.Malik: Galpha(q) and Gbetagamma regulate PAR-1 signaling of thrombin-induced NF-kappaB activation and ICAM-1 transcription in endothelial cells. *Circ Res* 91, 398-405 (2002)
68. Ahn H. S., C. Foster, G. Boykow, A. Stamford, M. Manna & M. Graziano: Inhibition of cellular action of thrombin by N3-cyclopropyl-7-((4-(1-methylethyl)phenyl)methyl)-7H-pyrrolo(3,2-f)quinazoline-1,3-diamine (SCH 79797), a nonpeptide thrombin receptor antagonist. *Biochem Pharmacol* 60, 1425-1434 (2000)
69. Arisato T., K, P. Sarker, K. Kawahara, M. Nakata, T. Hashiguchi, M. Osame, I. Kitajima & I. Maruyama: The agonist of the protease-activated receptor-1 (PAR) but not PAR3 mimics thrombin-induced vascular endothelial growth factor release in human smooth muscle cells. *Cell Mol Life Sci* 60, 1716-1724 (2003)
70. Stouffer, G. A. & M. S. Runge: The role of secondary growth factor production in thrombin-induced proliferation of vascular smooth muscle cells. *Semin Thromb Hemost* 24, 145-150 (1998)
71. Lee M. E., S. M. Kweon & S. U. Nham: Fibrin stimulates microfilament reorganization and IL-1 beta production in human monocytic THP-1 cells. *Mol Cell* 11, 13-20 (2001)
72. Lee M. E., K. J. Rhee & S. U. Nham: Fragment E derived from both fibrin and fibrinogen stimulates interleukin-6 production in rat peritoneal macrophages. *Mol Cell* 9, 7-13 (1999)
73. Liu X. & T. H. Piela-Smith: Fibrin(ogen)-induced expression of ICAM-1 and chemokines in human synovial fibroblasts. *J. Immunol* 165, 5255-5261 (2000)
74. Stouffer G A. & M. S. Runge: The role of secondary growth factor production in thrombin-induced proliferation of vascular smooth muscle cells. *Semin Thromb Hemost* 24, 145-150 (1998)
75. Szekanecz Z. & A. E. Koch: Vascular endothelium and immune responses: implications for inflammation and angiogenesis. *Rheum Dis Clin North Am* 30, 97-114 (2004)
76. Schmidt V.A., W. C. Nierman, D. R. Maglott, L. D. Cupit, K. A. Moskowitz, J. A. Wainer & W. F. Bahou: The human proteinase-activated receptor-3 (PAR-3) gene. Identification within a Par gene cluster and characterization in vascular endothelial cells and platelets. *J Biol Chem* 273, 15061-15068 (1998)
77. Libby, P. & D. I. Simon: Inflammation and thrombosis: The clot thickens. *Circulation* 103, 1718-1720 (2001)
78. Camerer E., J. A. Rottinhen, E. Gjernes, K. Larsen, A. H. Shartlien, J. G. Iversen & H. Prydz: Coagulation factors VIIa and Xa induce cell signaling leading to up-regulation of the egr-1 gene. *J Biol Chem* 274, 32225-32233 (1999)
79. Akahane K., K. Okamoto, M. Kikuchi, H. Todoroki, A. Higure, T. Ohuchida, K. Kitahara, S. Takeda, H. Itoh & K. Ohsato: Inhibition of factor Xa suppresses the expression of tissue factor in human monocytes and lipopolysaccharide-induced endotoxemia in rats. *Surgery* 130, 809-818 (2001)
80. Chen D., K. Riesbeck, J. H. McVey, G. Kemball-Cook, E. G. Tuddenham, R. I. Lechler & A. Dorling: Human thrombin and FXa mediate porcine endothelial cell activation; modulation by expression of TFPI-CD4 and hirudin-CD4 fusion proteins. *Xenotransplantation* 8, 258-265 (2001)
81. Langer F., C. Morys-Wortmann, B. Kusters & J. Storck: Endothelial protease-activated receptor-2 induces tissue factor expression and von Willebrand factor release. *Br J Haematol* 105, 542-550 (1999)
82. Haubitz M., M. Gerlach, H. J. Kruse & R. Brunkhorst: Endothelial tissue factor stimulation by proteinase 3 and elastase. Clin Exp Immunol 126, 584-588 (2001)
83. Scarpati E. M. & J. E. Sadler: Regulation of endothelial cell coagulant properties. Modulation of tissue factor, plasminogen activator inhibitors, and thrombomodulin by phorbol 12-myristate 13-acetate and tumor necrosis factor. *J. Biol Chem* 264, 20705-20713 (1989)
84. Grignani G. & A. Maiolo: Cytokines and hemostasis. *Haematologica* 85, 967-972 (2000)
85. Schecter A. D., B. J. Rollins, Y. J. Zhang, I. F. Charo, J. T. Fallon, M. Rossikhina, P. L. A. Giesen**,** Y. Nemerson & M. B. Taubman: Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. *J Biol Chem* 272, 28568-28573 (1997)
86. Collins P. W., K. E. Noble, J. R. Reittie, A. V. Hoffbrand, K. J. Pasi & K. L. Yong: Induction of tissue factor expression in human monocyte/endothelium cocultures. *Br J Haematol* 91, 963-970 (1995)
87. Celi A., G. Pellegrini, R. Lorenzet, A. De Blasi, N. Ready, B. C. Furie & B. Furie: P-selectin induces the expression of tissue factor on monocytes. *Proc Natl Acad Sci USA* 91, 8767-8771 (1994)
88. Mechtcheriakova D., G. Schabbauer, M. Lucerna, M. Clauss, R. De Martin, B. R. Binder & E. Hofer: Specificity, diversity, and convergence in VEGF and TNF-alpha signaling events leading to tissue factor up-regulation via EGR-1 in endothelial cells. *FASEB J* 15, 230-242 (2001)

89. Xuereb J. M., P. Sie, B. Boneu & J. Constans: Up-regulation of tissue factor expression by platelet-derived growth factor in human vascular smooth muscle cells in culture--role of mitogen-activated protein kinase and effects of intracellular cyclic AMP. *Thromb Haemost* 78, 1520-1526 (1997)
90. Caplice N. M., C. S. Mueske, L. S. Kleppe & R. D. Simari: Presence of tissue factor pathway inhibitor in human atherosclerotic plaques is associated with reduced tissue factor activity. *Circulation* 98, 1051-1057 (1998)
91. Miller D. L., K. Welty-Wolf, M. S. Carraway, M. Ezban, A. Ghio, H. Suliman & C. A. Piantadosi: Extrinsic coagulation blockade attenuates lung injury and proinflammatory cytokine release after intratracheal lipopolysaccharide. *Am J Respir Cell Mol Biol* 26, 650-658 (2002)
92. McLean K., S. Schirm, A. Johns, J. Morser & D. R. Light: FXa-induced responses in vascular wall cells are PAR-mediated and inhibited by ZK-807834. *Thromb Res* 103, 281-297 (2001)
93. Lindmark E. & A. Siegbahn: Tissue factor regulation and cytokine expression in monocyte-endothelial cell co-cultures. Effects of a statin, an ACE-inhibitor and a low-molecular-weight heparin. *Thromb Res* 108, 77-84 (2002)
94. Pernerstorfer T., U. Hollenstein, J. Hansen, M. Knechtelsdorfer, P. Stohlawetz, W. Graninger, H.-G. Eichler, W. Speiser & B. Jilma: Heparin blunts endotoxin-induced coagulation activation. *Circulation* 100, 2485-2490 (1999)
95. Gertz S. D., J. T. Fallon, R. Gallo, M. B. Taubman, S. Banai, W. L. Barry, L. W. Gimple, Y. Nemerson, S. Thiruvikraman, S. S. Naidu, J. H. Chesebro, V. Fuster, I. J. Sarembock & J. J. Badimon: Hirudin reduces tissue factor expression in neointima after ballon injury in rabbit femoral and porcine coronary arteies. *Circulation* 98, 580-587 (1998)
96. Chen X., S. Ren, M. G. Ma, S. Dharmalingam, L. Lu, M. Xue, J. Ducas & G. X. Shen: Hirulog-like peptide reduces restenosis and expression of tissue factor and transforming growth factor-beta in carotid artery of atherosclerotic rabbits. *Atherosclerosis* 169, 31-40. (2003)
97. Souter PJ, Thomas S, Hubbard AR, Poole S, Romisch J, Gray E. (2001) Antithrombin inhibits lipopolysaccharide-induced tissue factor and interleukin-6 production by mononuclear cells, human umbilical vein endothelial cells, and whole blood. *Crit Care Med* 29, 134-9.
98. Hou L., G. L. Howells, S. Kapas & M. G. Macey: The protease-activated receptors and their cellular expression and function in blood-related cells. *Br J Haematol* 101, 1-9 (1998)
99. Henke P. K., T. W. Wakefield, A. M. Kadell, M. J. Linn, M. R. Varma, M. Sarkar, A. Hawley, J. B. Fowlkes & R. M. Strieter: Interleukin-8 administration enhances venous thrombosis resolution in a rat model. *J Surg Res* 99, 84-91 (2001)
100. Thanaporn P., D. D. Myers, S. K. Wrobleski, A. E. Hawley, D. M. Farris, T. W. Wakefield & P. J. Henke: P-selectin inhibition decreases post-thrombotic vein wall fibrosis in a rat model. *Surgery* 134, 365-371 (2003)
101. Palabrica T., R. Lobb, B. C. Furie, M. Aronovitz, C. Benjamin, Y. M. Hsu, S. A. Sajer & B. Furie: Leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets. *Nature* 359(6398), 848-851 (1992)
102. Murphy J. F. & J.L. McGregor: A peptide (P2) derived from the variable heavy chain of an anti-P-selectin monoclonal antibody (LYP20) inhibits leucocyte adhesion to thrombin-activated platelets and endothelial cells. *Br J Haematol* 120, 605-610 (2003)
103. Toombs C. F., G. L. DeGraaf, J. P. Martin, J. G. Geng, D. C. Anderson & R. J. Shebuski: Pretreatment with a blocking monoclonal antibody to P-selectin accelerates pharmacological thrombolysis in a primate model of arterial thrombosis. *J Pharmacol Exp Ther* 275, 941-949 (1995)
104. Wakefield T. W., R. M. Strieter, C. A. Wilke, A. M. Kadell, S. K. Wrobleski, M. D. Burdick, R. Schmidt, S. L. Kunkel, & L. J. Greenfield: Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. *Arterioscler Thromb Vasc Biol* 15, 258-268 (1995)
105. Selheim F., H. Holmsen & F. S. Vassbotn: Identification of functional VEGF receptors on human platelets. *FEBS Lett* 512, 107-110 (2002)
106. Tan P., F. W. Luscinskas & S. Homer-Vanniasinkam: Cellular and molecular mechanisms of inflammation and thrombosis. *Eur J Vasc Surg* 17, 373-389 (1999)
107. Leytin V., S. Shakoor, M. Mody, D. Allen, B. Garvey & J. Freedman: Sepsis- and endotoxemia-generated cytokines do not trigger activation of human platelets. *Crit. Care Med*. 30, 2771-2773 (2002)
108. Hayden M., M. Pignone, C. Phillips & C. Mulrow: Aspirin for the primary prevention of cardiovascular events: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 136, 161-172 (2002)
109. McKenzie M. E., A. I. Malinin, C. R. Bell, A. Dzhanashvili, E. D. Horowitz, B. R. Oshrine, D. Atar & V. L. Serebruany Aspirin inhibits surface glycoprotein IIb/IIIa, P-selectin, CD63, and CD107a receptor expression on human platelets. *Blood Coagul Fibrinolysis* 14, 249-253 (2003)
110. Pitt B., C. Pepine & J. T. Willerson: Cyclooxygenase-2 inhibition and cardiovascular events. *Circulation* 106, 167-169 (2002)
111. de Gaetano G., M. B. Donati & C. Cerletti: Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors. *Trends Pharmacol Sci* 24, 245-252 (2003)
112. Cheung W. M., M. R. D'Andrea, P. Andrade-Gordon & B. P. Damiano: Altered vascular injury responses in mice deficient in protease-activated receptor-1. *Arterioscler Thromb Vasc Biol* 19, 3014-3024 (1999)
113. Major C. D., R. J. Santulli, C. K. Derian & P. Andrade-Gordon: Extracellular mediators in atherosclerosis and thrombosis: lessons from thrombin receptor knockout mice. *Arterioscler Thromb Vasc Biol* 23, 931-939 (2003)
114. Matsuyama M., K. Akioka, M. Okamoto, Y. Kadotani, K. Nakamura, H. Ushigome, Y. Ohmori, R. Yoshimura, T. Nakatani & N. Yoshimura: Control of renal ischemic reperfusion injury by antisense oligodeoxynuceotide of tissue factor. *Tansplant Proc* 35, 103-104 (2003)
115. Ghrib F., P.Leger, M. Ezban, A. Kristensen, J. Cambus & B. Boneu: Anti-thrombotic and haemorrhagic effects of active site-inhibited factor VIIa in rats. *Br J Haematol* 112, 506-512 (2001)

116. Lev E. I., J. D. Mamur, M. Zdravkovic, J. I. Osende, J. Robbins, J. A. Delfin, M. Richard, E. Erhardtsen, M. S. Thomsen, A. M. Lincoff & J. J. Badimon: Antithrombotic effect of tissue factor inhibition by inactivated Factor VIIa. *Arterioscler Thromb Vasc Biol* 22, 1036-1041 (2002)
117. Orvim U., R. M. Barstad, L. Orning, L. B. Petersen, M. Ezban, U. Hedner & K. S. Sakariassen: Antithrombotic efficacy of inactivated active site recombinant factor VIIa is shear dependent in human blood. *Arterioscler Thromb Vasc Biol* 17, 3049-3056 (1997)
118. Kirchhofer D., T. B. Tschopp & H. R.Baumgartner: Active site-blocked factors VIIa and IXa differentially inhibit fibrin formation in a human *ex vivo* thrombosis model. *Arterioscler Thromb Vasc Biol* 15, 1098-1106 (1995)
119. Himber J., C. J. Refino, L. Burcklen, S. Roux & D. Kirchhofer: Inhibition of arterial thrombosis by a soluble tissue factor mutant and active site-blocked factors IXa and Xa in the guinea pig. *Thromb Haemost* 85, 475-481 (2001)
120. Orning L., P. M.Fischer, C. K. Hu, E. Agner, M. Engebretsen, M. Husbyn, L B. Petersen, U. Orvim, M. Llinas & K. S. Sakariassen: A cyclic pentapeptide derived from the second EGF-like domain of Factor VII is an inhibitor of tissue factor dependent coagulation and thrombus formation. *Thromb Haemost* 87, 13-21 (2002)
121. Szalony J. A., O. D. Suleymanov, A. K. Salyers, S. G. Panzer-Knodle, J. D. Blom, R. M. LaChance, B. L. Case, J. J. Parlow, M. S. South, R. S. Wood & N. S. Nicholson: Administration of a small molecule tissue factor/factor VIIa inhibitor in a non-human primate thrombosis model of venous thrombosis: effects on thrombus formation and bleeding time. *Thromb Res* 112, 167-174 (2003)
122. Nijkeuter M. & M. V. Huisman: Pentasaccharides in the prophylaxis and treatment of venous thromboembolism: a systematic review. *Curr Opin Pulm Med* 10, 338-344 (2004)
123. Bergqvist D.: Enoxaparin: a pharmacoeconomic review of its use in the prevention and treatment of venous thromboembolism and in acute coronary syndromes. *Pharmacoeconomics* 20, 225-243 (2002)
124. Chapman T. M. & K. L. Goa: Bemiparin: a review of its use in the prevention of venous thromboembolism and treatment of deep vein thrombosis. *Drugs* 63, 2357-2377 (2003)
125. Wellington K., K. McClellan & B. Jarvis: Reviparin: a review of its efficacy in the prevention and treatment of venous thromboembolism. *Drugs* 61, 1185-1209 (2001)
126. Diquelou A., D. Dupouy, R. Cariou, K. S. Sakariassen, B. Boneu & Y. Cadroy: A comparative study of the anticoagulant and anti-thrombotic effects of unfractionated heparin and a low molecular weight heparin (Fraxiparine) in an experimental model of human venous thrombosis. *Thromb Haemost* 74, 1286-1292 (1995)
127. Cheer S. M., C. J. Dunn & R. Foster: Tinzaparin sodium: a review of its pharmacology and clinical use in the prophylaxis and treatment of thromboembolic disease. *Drugs* 64, 1479-1502 (2004)
128. Pineo G. F. & R. D. Hull: Dalteparin sodium. *Expert Opin Pharmacother* 2, 1325-1337 (2001)
129. Bal dit Sollier C., C. Kang, N. Berge, J. P. Herault, M. Bonneau, J. M. Herbert & L. Drouet: Activity of a synthetic hexadecasaccharide (SanOrg123781A) in a pig model of arterial thrombosis. *J Thromb Haemost* 2, 925-930 (2004)
130. Ragosta M., L. W. Gimple, S. D. Gertz, C. T. Dunwiddie, G. P. Vlasuk, H. L. Haber, E. R. Powers, W. C. Roberts & I. J. Sarembock: Specific factor Xa inhibition reduces restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits. *Circulation* 89, 1262-1271 (1994)
131. Orvim U., R. M. Barstad, G. P. Vlasuk & K. S. Sakariassen: Effect of selective factor Xa inhibition on arterial thrombus formation triggered by tissue factor/factor VIIa or collagen in an *ex vivo* model of shear-dependent human thrombogenesis. *Arterioscler Thromb Vasc Biol* 15, 2188-2194 (1995)
132. Kaiser B., W. Jeske, J. M. Walenga & J. Fareed: Inactivation of factor Xa by the synthetic inhibitor DX-9065a causes strong anticoagulant and antiplatelet actions in human blood. *Blood Coagul Fibrinolysis* 10, 495-501 (1999)
133. Iba T., A. Kidokoro, M. Fukunaga, S. Fuse, M. Suda, S. Kunitada & T. Hara: Factor Xa-inhibitor (DX-9065a) modulates the leukocyte-endothelial cell interaction in endotoxemic rat. *Shock* **17**, 159-162 (2002)
134. Yokoyama T., A. B.Kelly, U. M. Marzec, S. R. Hanson, S. Kunitada & L. A. Harker: Antithrombotic effects of orally active synthetic antagonist of activated factor X in nonhuman primates. *Circulation* 92, 485-491(1995)
135. Abendschein D. R., P. K. Baum, D. R. Light & J. Morser: Effect of vascular injury on inhibition of venous thrombosis with ZK-807834, a direct inhibitor of factor Xa. *J Thromb Haemost* 1, 1955-1958 (2003)
136. McClanahan T. B., G. W. Hicks, A. L. Morrison, Y. W. Peng, N. Janiczek-Dolphin, T. E. Mertz, M. E. Sullivan, J. Morser, P L. Juneau & R. Leadley: The antithrombotic effects of CI-1031 (ZK-807834) and enoxaparin in a canine electrolytic injury model of arterial and venous thrombosis. *Eur J Pharmacol* 432, 187-194 (2001)
137. Wong P. C., M. L. Quan, E. J. Crain, C. A. Watson, R. R. Wexler & R. M. Knabb: Nonpeptide factor Xa inhibitors: I. Studies with SF303 and SK549, a new class of potent antithrombotics. *J Pharmacol Exp Ther* 292, 351-357 (2000)
138. Sato K., Y. Taniuchi, T. Kawasaki, F. Hirayama, H. Koshio, Y. Matsumoto & Y. Iizumi: Comparison of the anticoagulant and antithrombotic effects of YM-75466, a novel orally-active factor Xa inhibitor, and warfarin in mice. *Jpn J Pharmacol* 78, 191-197 (1998)
139. Rebello S. S., R. G. Bentley, S. R. Morgan, C. J. Kasiewski, V. Chu, M. H. Perrone & R. J. Leadley: Antithrombotic efficacy of a novel factor Xa inhibitor, FXV673, in a canine model of coronary artery thrombolysis.
Br J Pharmacol 133, 1190-1198 (2001)
140. Wong P. C., E. J. Crain, C. A. Watson, A. M. Zaspel, M. R. Wright, P.Y. Lam, D. J. Pinto, R. R. Wexler & R. M. Knabb: Nonpeptide factor Xa inhibitors III: effects of DPC423, an orally-active pyrazole antithrombotic agent, on arterial thrombosis in rabbits. *J Pharmacol Exp Ther* 303, 993-1000 (2002)
141. Pruitt J. R., D. J. Pinto, M J. Estrella, L. L. Bostrom, R. M. Knabb, P. C. Wong, M.R. Wright & R. R. Wexler: Isoxazolines and isoxazoles as factor Xa inhibitors. *Bioorg Med Chem Lett* 10, 685-689 (2000)

142. Bostwick J. S., R. Bentley, S. Morgan, K. Brown, V. Chu, W. R. Ewing, A. P. Spada, H. Pauls, M. H. Perrone, C. T. Dunwiddie & R. J. Jr. Leadley: RPR120844, a novel, specific inhibitor of coagulation factor Xa inhibits venous thrombosis in the rabbit. *Thromb Haemost* 81, 157-160 (1999)
143. Nutescu E., M. Singh-Khalsa; Heparin Consensus Group: Treatment of venous thromboembolism: challenging the unfractionated heparin standard. *Pharmacotherapy* 24(8 Pt 2), 127S-131S (2004)
144. Fischer K. G.: The role of recombinant hirudins in the management of thrombotic disorders. *BioDrugs* 18, 235-268 (2004)
145. Dager W. E., T. G. Vondracek, B. A. McIntosh & E. A.Nutescu: Ximelagatran: An Oral Direct Thrombin Inhibitor (CE) *Ann Pharmacother* 38, 1881-1897(2004)
146. Fareed J. & W. P. Jeske: Small-molecule direct antithrombins: argatroban. *Best Pract Res Clin Haematol* 17, 127-138 (2004)
147. Vogel G. M., D. G. Meuleman, T. G. Van Dinther, R. Buijsman, A. W. Princen & M. J. Smit: Antithrombotic properties of a direct thrombin inhibitor with a prolonged half-life and AT-mediated factor Xa inhibitory activity. *J Thromb Haemost* 1, 1945-1954 (2003)
148. Lindahl A. K., P. M. Sandset, M. Thune-Wiiger, O. Nordfang & K. S. Sakariassen: Tissue factor pathway inhibitor prevents thrombus formation on procoagulant subendothelial matrix. *Blood Coagul Fibrinolysis* 5, 755-760 (1994)
149. Holst J., B. Lindblad, D. Bergqvist, O. Nordfang, P. B. Ostergaard, J. G. Petersen, G. Nielsen & U. Hedner: Antithrombotic effect of recombinant truncated tissue factor pathway inhibitor (TFPI1-161) in experimental venous thrombosis--a comparison with low molecular weight heparin. *Thromb Haemost* 71, 214-219 (1994)
150. Sheppard D. R.: Activated protein C resistance: the most common risk factor for venous thromboembolism. *J Am Board Fam Pract* 13, 111-115 (2000)
151. Bhattacharyya M., M. Kannan, V. P. Chaudhry & R. Saxena: Venous thrombosis: prevalence of prothrombotic defects in north Indian population. *Indian J Pathol Microbiol* 46, 621-624 (2003)
152. Espana F., A. Vaya, Y. Mira, P. Medina, A. Estelles, P. Villa, C. Falco & J. Aznar: Low level of circulating activated protein C is a risk factor for venous thromboembolism. *Thromb Haemost* 86, 1368-1373 (2001)
153. Urano T., F. J. Castellino, H. Ihara, Y. Suzuki, M. Ohta, K. Suzuki & H. Mogami: Activated protein C attenuates coagulation-associated over-expression of fibrinolytic activity by suppressing the thrombin-dependent inactivation of PAI-1. *J Thromb Haemost* 1, 2615-2620 (2003)
154. Bajzar L., M.E. Nesheim & P. B. Tracy: The profibrinolytic effect of activated protein C in clots formed from plasma is TAFI-dependent. *Blood* 88, 2093-2100 (1996)
155. Jackson C. V., B. D. Bailey & T. J. Shetler: Pharmacological profile of recombinant, human activated protein C (LY203638) in a canine model of coronary artery thrombosis. *J Pharmacol Exp Ther* 295, 967-971 (2000)
156. Aoki Y., Y. Fukumoto, K. Inoue, Y. Katsuura, K. Komoriya & S. Miyamoto: Effect of activated human protein C on experimental venous thrombosis induced by stasis with operative invasion in mice. *Arzneimittelforschung* 50, 695-699 (2000)
157. Kurz K. D., T. Smith, A. Wilson, B. Gerlitz, M. A. Richardson & B. W.Grinnell: Antithrombotic efficacy in the guinea pig of a derivative of human protein C with enhanced activation by thrombin. *Blood* 89, 534-540 (1997)
158. Araki H., K. Nishi, N. Ishihara & K. Okajima: Inhibitory effects of activated protein C and heparin on thrombotic arterial occlusion in rat mesenteric arteries. *Thromb Res* 62, 209-216 (1991)
159. Gruber A., S. R. Hanson, A. B. Kelly, B. S. Yan, N. Bang, J. H. Griffin & L. A. Harker: Inhibition of thrombus formation by activated recombinant protein C in a primate model of arterial thrombosis. *Circulation* 82, 578-585 (1990)
160. Malm K., B. Dahlback & B. Arnljots: Prevention of thrombosis following deep arterial injury in rats by bovine activated protein C requiring co-administration of bovine protein S. *Thromb Haemost* 90, 227-234 (2003)
161. Arnljots B., D. Bergqvist & B. Dahlback: Inhibition of microarterial thrombosis by activated protein C in a rabbit model. *Thromb Haemost* 72, 415-420 (1994)
162. Foo R. S., A. H. Gershlick, K. Hogrefe, J. H. Baron, T. W. Johnston, A. J. Hussey, I. Garner & D. P. de Bono: Inhibition of platelet thrombosis using an activated protein C-loaded stent: *in vitro* and *in vivo* results. *Thromb Haemost* 83, 496-502 (2000)
163. Ostrovsky L., R. C. Woodman, D. Payne, D. Teoh & P. Kubes: Antithrombin III prevents and rapidly reverses leukocyte recruitment in ischemia/reperfusion. *Circulation* 96, 2302-2310 (1997)
164. Bauer K. A.: Low intensity warfarin: is it clinically useful in venous thromboembolism management? *Br J Haematol* 127, 155-158 (2004)
165. Nose T., Y. Shimohigashi, M. Ohno, T. Costa, N. Shimizu & Y. Ogino: Enhancement of thrombin receptor activation by thrombin receptor-derived heptapeptide with parafluorophenylalanine in place of phenylalalnine. *Biochem Biophys Res Commun* 193, 694-699 (1993)
166. Elliott J. T., W. J. Hoekstra, B. E. Maryanoff & G. D. Prestwich: Photoactivatable peptides based on BMS-197525: A potent antagonist of the human thrombin receptor (PAR-1) *Bioorg Med Chem Lett* 9, 279-284 (1999)
167. Damiano B. P., C. K. Derian, B. E. Maryanoff, H. C. Zhang & P. A.Gordon: RWJ-58259: a selective antagonist of protease activated receptor-1. *Cardiovasc Drug Rev* 21, 313-326 (2003)
168. Derian C. K., B. P. Damiano, M. F. Addo, A. L. Darrow, M. R. D'Andrea, M. Nedelman, H. C. Zhang, B. E. Maryanoff & P. Andrade-Gordon: Blockade of the thrombin receptor protease-activated receptor-1 with a small-molecule antagonist prevents thrombus formation and vascular occlusion in nonhuman primates. *J Pharmacol Exp Ther* 304, 855-861 (2003)
169. Andrade-Gordon P., C. K. Derian, B. E. Maryanoff, H. C. Zhang, M. F. Addo, W. M. Cheung, B. P. Damiano, M. R. D'Andrea, A. L. L. Darrow, de Garavilla, A.J. Eckardt, E. C. Giardino, B. J. Haertlein & D. F. McComsey: Administration of a potent antagonist of protease-activated receptor-1 (PAR-1) attenuates vascular

restenosis following balloon angioplasty in rats. *J Pharmacol Exp Ther* 298, 34-42 (2001)
170. Kato Y., Y. Kita, Y. Hirasawa-Taniyama, M. Nishio, K. Mihara, K. Ito, T. Yamanaka, J. Seki, S. Miyata & S. Mutoh: Inhibition of arterial thrombosis by a protease-activated receptor 1 antagonist, FR171113, in the guinea pig. *Eur J Pharmacol* 473, 163-169 (2003)
171. Wu C. C., T. L. Hwang, C. H. Liao, S. C. Kuo, F. Y. Lee, C. Y. Lee & C. M. Teng: Selective inhibition of protease-activated receptor 4-dependent platelet activation by YD-3. *Thromb Haemost* 87, 1026-1033 (2000)
172. Takada M., H. Tanaka, T. Yamada, O. Ito, M. Kogushi, M. Yanagimachi, T. Kawamura, T. Musha, F. Yoshida, M. Ito, H. Kobayashi, S. Yoshitake & I. Saito: Antibody to thrombin receptor inhibits neointimal smooth muscle cell accumulation without causing inhibition of platelet aggregation or altering hemostatic parameters after angioplasty in rat. *Cir Res* 82, 980-987 (1998)
173. Cook J. J., G. R. Sitko, B. Bednar, C. Condra, M.J. Mellott, D.M. Feng, R. F. Nutt, J. A. Shafer, R. J. Gould & T. M. Connolly: An antibody against the exosite of the coned thrombin receptor inhibits experiemental arterial thrombosis in the African green monkey. *Circulation* 9, 2961-2971 (1995)
174. Willerson J.T & P. M. Ridker: Inflammation as a cardiovascular risk factor. *Circulation* 109(S II), II2-10 (2004)
175. Holvoet P. & D. Collen: Thrombosis and atherosclerosis. *Curr Opin Lipidol* 8, 320-328 (1997)
176. Fareed J., D. A. Hoppensteadt & R. L. Bick: Management of thrombotic and cardiovascular disorders in the new millenium. *Clin Appl Thromb Hemost* 9, 101-108 (2003)
177. Sorensen B. B., U. Hedner & E. Erhardtsen: rFVIIai in Acute Coronary Syndromes. *Semin Vasc Med* 3,199-204 (2003)
178. Montalescot G., J. P. Collet, M. L. Tanguy, A. Ankri, L. Payot, R. Dumaine, R. Choussat, F. Beygui, V. Gallois & D. Thomas: Anti-Xa activity relates to survival and efficacy in unselected acute coronary syndrome patients treated with enoxaparin. *Circulation* 110, 392-398 (2004)
179. Chen A. A. & M. S.Sabatine: The management of unstable angina and non-ST-segment elevation myocardial infartion. *Minerva Cardioangiol* 51, 433-445 (2003)
180. Yan A.T. & S. G.Goodman: Low-molecular-weight heparins in ischemic heart disease. *Curr Opin Cardiol* 19, 309-316 (2004)
181. Petersen J.L., K. W. Mahaffey, V. Hasselblad, E. M. Antman, M. Cohen, S. G. Goodman, A. Langer, M. A. Blazing, A. Le-Moigne-Amrani, J. A. de Lemos, C. C. Nessel, R. A. Harrington, J. J. Ferguson, E. Braunwald & R. M. Califf: Efficacy and bleeding complications among patients randomized to enoxaparin or unfractionated heparin for antithrombin therapy in non-ST-Segment elevation acute coronary syndromes: a systematic overview. *JAMA* 292, 89-96 (2004)
182. Lincoff A. M., J. A. Bittl, N. S. Kleiman, I. J. Sarembock, J. D. Jackman, S. Mehta, M. A. Tannenbaum, A. L. Niederman, W. B. Bachinsky, J. Tift-Mann 3rd, H. G. Parker, D. J. Kereiakes, R. A. Harrington, F. Feit, E. S. Maierson, D. P. Chew & E. J. Topol: REPLACE-1 Investigators: Comparison of bivalirudin versus heparin during percutaneous coronary intervention (the Randomized Evaluation of PCI Linking Angiomax to Reduced Clinical Events (REPLACE)-1 trial) *Am J Cardiol* 93, 1092-1096 (2004)
183. Bates E. R.: Bivalirudin: an anticoagulant option for percutaneous. *Expert Rev Cardiovasc Ther* 2, 153–162 (2004)
184. Carswell C. I. & G. L. Plosker: Bivalirudin: A Review of its Potential Place in the Management of Acute Coronary Syndromes. *Drugs* 62, 841-870 (2002)
185. Hofmann T.: Clinical application of enoxaparin. *Expert Rev Cardiovasc Ther* 2, 321–337 (2004)
186. Halperin J.L.: Ximelagatran: oral direct thrombin inhibition as anticoagulant therapy in atrial fibrillation. *J Am Coll Cardiol* 45, 1-9 (2005)
187. Harrigan M. R., E. I. Levy, B. R. Bendok & L. N. Hopkins: Bivalirudin for endovascular intervention in acute ischemic stroke: case report. *Neurosurgery* 54, 218-222 (2004)
188. Sinnaeve P. R. & F. J. Van de Werf: Will oral antithrombin agents replace warfarin? The new oral direct thrombin inhibitor ximelagatran is at least equivalent to warfarin for stroke prevention in patients with non-valvar atrial fibrillation, and seems to be a promising adjunct to aspirin after acute coronary syndrome. *Heart* 90, 827-828 (2004)
189. Chackalamannil S.: G-protein coupled receptor antagonists-1: protease activated receptor-1 (PAR-1) antagonists as novel cardiovascular therapeutic agents. *Curr Top Med Chem* 3, 1115-2113 (2003)
190. McGuire J. J.: Proteinase-activated Receptor 2 (PAR2): a challenging new target for treatment of vascular diseases. *Curr Pharm Des* 10, 2769-2778 (2004)

Abbreviations: AAPPA: amidinoaryl propanoic acid, ACS: acute coronary syndrome, APC: activated protein C, AT III: antithrombin III , ATS: antistasin, Cox-1(2): cycloxygenase-1(2), CRP: C-reactive protein, DVT: deep vein thrombosis , FIIa: thrombin, FVIIa: activated factor VII, FVIIai: active-site inhibited FVIIa, FXa: activated factor X, GP: glycoprotein , ICAM: Intracellular adhesion molecule, IL: interleukin, LMWH: low molecular weight heparin, LPS: lipopolysaccharide; bacterial endotoxin, MCP: monocyte chemotractic protein, MI: myocardial infraction , PAI-1: plasminogen activator inhibitor-1, PAR: protease activated receptor, PCI: percutaneous coronary intervention , PDGF: platelet derived growth factor, PL: phospholipids, SMC: smooth muscle cell, TF: tissue factor , TFPI: TF pathway inhibitor , TNF: tissue necrosis factor-alpha, TAFI: thrombin-activatable fibrinolytic inhibitor, TAP: tick anticoagulant protein , VCAM: vascular adhesion molecule, VEC: vascular endothelial cell, VEGF: vascular endothelial growth factor , VTE: venous thromboembolism

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