Tissue Factor in the Pathogenesis of Atherosclerosis

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Introduction

Thrombosis is integral to the development and progression and clinical sequelae of atherosclerosis. Acute thrombosis can occur spontaneously, leading to catastrophic arterial occlusion and resulting in myocardial infarction, unstable angina, stroke, and sudden death (1,2). Acute thrombosis also occurs as a complication of arterial bypass surgery, balloon angioplasty, atherectomy, or coronary artery stenting (3–5).

Non-occlusive thrombus is an important component of the atherosclerotic plaque. Thrombus is comprised principally of platelets and fibrin. White blood cells and circulating proteins are also trapped within the platelet-fibrin thrombus. Activated platelets release a variety of growth factors and cytokines that have been implicated in vessel inflammation and in vascular smooth muscle cell (SMC) proliferation and migration (6,7). Thrombin (8) and factor X/Xa (9,10) also have direct effects on SMC and may play a role in the development of intimal hyperplasia. In addition, products secreted by leukocytes trapped within the thrombus may have direct effects on the vessel wall.

Tissue factor (TF) is a membrane-bound glycoprotein that initiates coagulation (11,12). Human TF consists of three domains: a short cytoplasmic domain of 19 residues, a single transmembrane domain of 23 residues, and a large extracellular domain of 219 residues. In addition, there is a 32 residue amino-terminal leader sequence which is cleaved to produce the mature molecule. TF binds to factor VII/VIIa, and the resulting complex acts as a catalyst for the conversion of factors IX and X to IXa and Xa respectively, triggering the clotting cascade. This ultimately leads to the generation of thrombin, which in turn cleaves fibrinogen to fibrin, the major ingredient of the thrombus. Recent studies suggest that the accumulation of TF in atherosclerotic plaques plays a major role in determining plaque thrombogenicity. TF is also rapidly induced in the vessel wall as a consequence of acute arterial injury. Both phenomena may be important in the thrombotic complications of atherosclerotic heart disease.

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TF in atherosclerotic plaques

Plaque rupture is thought to be a major precipitant of acute arterial thrombosis (1,2,13), exposing circulating blood to thrombogenic plaque proteins. Rupture often occurs at fissures in "unstable" lipid-rich lesions which appear non-obstructive on coronary angiography. Thrombosis also occurs on severely stenotic vessels possessing heavily calcified, "stable" plaques. Trauma to the surface of these stenotic plaques may induce *de novo* synthesis of procoagulants or expose pre-existing molecules to the circulation. The increased wall shear stress seen in severely stenotic vessels may be a contributing factor.

TF mRNA and antigen has been found in abundance in the adventitia of normal human coronary and internal mammary arteries and aortas (14,15). In contrast, TF mRNA and antigen have been undetectable in normal arterial endothelium and either undetectable or present at low levels in the media of coronary and internal mammary arteries (14,15). Zitlick et al. (57) initially identified TF antigen in both cellular and acellular components of human atherosclerotic plaques (57). In a study of atherosclerotic plaques from carotid endarterectomy specimens (15), TF mRNA and protein were absent from the endothelium, but were identified in mesenchymal-like intimal cells (presumably SMC) as well as in foam cells and monocytes adjacent to cholesterol clefts, and in the extracellular matrix (15). Annex and coworkers (16) found TF antigen present in 33% of *de novo* lesions (n=43) and in 6% of restenotic lesions (n=18) from human coronary atherectomy specimens.

We have recently examined TF procoagulant activity in 63 coronary atherectomy specimens (17). Significant TF activity was detected in 53/63 (84%). TF antigen was detected in 43/50 lesions examined (86%), and was expressed in cellular and acellular areas of the plaque. Thrombus was present in 19/43 lesions with detectable TF antigen, in contrast to 0/7 lesions without detectable TF antigen ($p<0.02$). Moreno and colleagues (18) also detected TF antigen in virtually all samples from 50 coronary atherectomies. Using a semi-quantitative morphometric analysis, TF content (expressed as percentage of sample containing TF) was found to be larger in samples from patients with unstable angina (42±3%) than stable angina (18±4%). They also noted that TF predominated in cellular areas containing macrophages and SMC in patients with unstable angina, whereas TF was found mostly

in acellular areas in patients with stable angina. The higher detection rate in these series of atherectomy specimens may be due to differences in the sensitivity of the TF antibodies or to differences in the patient populations. These factors may also account for the more diffuse immunohistochemical localization of TF antigen compared with those previously reported.

In our study of coronary atherectomies (17), some specimens with predominantly extracellular staining on immunohistochemistry demonstrated high levels of TF activity. Because these studies were performed only on small fragments, we examined TF expression in atherosclerotic human coronary and carotid arteries. Studies involved the use of antibodies to TF as well as a highly sensitive binding assay employing digoxigenin-labeled Factors VIIa and X (19). TF was detected in all (fifty) atherosclerotic plaques using either technique. Of particular note was the intense staining of the lipid-rich core (1). TF staining and digoxigenin-labeled VIIa binding were also noted in macrophages surrounding the core and in plaque SMC, as well as in relatively acellular, fibrotic regions of advanced plaques. The arterial media showed staining of only rare SMC. The endothelium overlying the plaque also stained for TF.

The finding of abundant TF in the lipid-rich core is consistent with studies performed by Badimon and co-workers in which plaque components (normal intima, fatty streak, fibro-lipid and sclerotic plaques and lipid-rich atheromatous core with cholesterol crystals) were placed in a perfusion chamber (20) and exposed to circulating blood at high shear rates. Thrombus formation and platelet deposition were then examined. The lipid rich core was found to be the most thrombogenic component (21–23). The etiology of the TF in the lipid-rich core and in the extracellular matrix of acellular, fibrotic regions remains to be determined. However, the above studies suggest that this TF may be biologically active. This extracellular TF may be released from the surface of living cells (24) or may be derived from cell debris. Based upon immunohistochemical data, the macrophage would appear to be the predominant source.

TF in arterial injury

Thrombosis is commonly associated with acute arterial injury, such as that produced by coronary angioplasty, directional atherectomy, and coronary artery stenting (3–5). Thrombus formation after vessel injury involves adherence of platelets to the subendothelium and activation of the coagulation cascade, presumably by exposure of circulating clotting factors to procoagulants within the vessel wall.

A variety of animal models have been employed to examine thrombus formation in acute arterial injury. In the pig carotid balloon injury model, superficial injury (endothelial denudation without medial injury) is associated with platelet deposition but no fibrin generation. Deeper injury (manifested by a medial tear) results in marked platelet accumulation and fibrin generation (25). Balloon injury to normal rodent arteries does not result in fibrin deposition, even when medial smooth muscle injury is present (26–28). In normal rabbit and rat aorta, endothelial denudation with a balloon catheter results in the rapid deposition of a monolayer of platelets with no associated fibrin formation (26–28). In contrast, fibrin deposition occurs rapidly when previously injured rabbit arteries, possessing a neointima, are subjected to a second injury (26,28,29).

We have recently examined TF expression and fibrin deposition in rat aortic and carotid arteries using single and double injury models. Fibrinogen was seen on the luminal surface after the first and second injury. Fibrin deposition and microthrombi were not seen at any time after single injury, but were present on the luminal surface following the second injury. In uninjured rat aortic media, TF mRNA was

Fig. 1. Expression of TF in human coronary atherosclerotic plaque. Paraffin section was stained with digoxigenin-labeled factor VIIa as described (19). TF (dark staining)) is present extracellularly in the lipid rich core surrounding cholesterol clefts. Staining is also present in the fibrous cap and in the adventitia. (Original magnification x100). NC = necrotic core. The lumen contains dye from postmortem angiography.

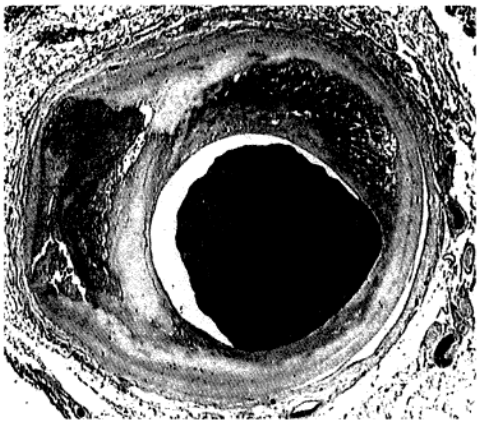

Fig. 2. Expression of TF in rat neointima. Rat aortas were subjected to balloon arterial injury as described (30). Two weeks after injury, the aortas were harvested and paraffin sections stained with antibody to soluble tissue factor (sTF) (described in 19). TF (dark staining) is abundant in the intima (I), with minimal staining seen in the media (M). (Original magnification x 400).

undetectable by blot or *in situ* hybridization. Two hours after balloon injury, medial TF mRNA levels increased markedly, returning to baseline after 24 hours (30). TF antigen and Factor VIIa (FVIIa) binding were not detectable in the endothelium or media of normal vessels or during the first 4 hours after injury. In contrast, medial TF was abundant at 24 hours and then slowly returned to baseline. Subsequently, TF antigen accumulated in the developing intimal plaque, with high levels detectable 2 weeks after single injury (Fig. 2). Staining of whole mount preparations showed minimal TF antigen on the surface of uninjured or singly-injured vessels. In contrast, the second injury was associated with rapid and marked exposure (within 5 min) of TF antigen.

These studies suggest that the initial response of normal arteries to injury is the rapid transcription of TF in the medial SMC. TF protein is expressed transiently in the media, but is protected from the circulation by the presence of an intact internal elastic lamina and by the rapid deposition of a platelet monolayer on the luminal surface. The migration of SMC from the media to the intima and the subsequent proliferation of intimal SMC is accompanied by the pronounced accumulation of TF in the intima. This TF is also protected from the circulation by platelets, components of the extracellular matrix, and regenerating endothelium. The second injury denudes the endothelium and subendothelial matrix and exposes active intimal TF to circulating blood, leading to activation of coagulation and fibrin deposition.

Induction of TF in SMC near the luminal border has also been reported in rabbit (31,32) and porcine (33) models of arterial injury. The significance of TF induction in acute injury remains to be determined. In 9 rabbits subjected to arterial injury and mechanical stenosis, antibodies to TF inhibited the variations in cyclical flow (31), thought to result in part from platelet thrombus formation and dislodgment. TF antibody also inhibited thrombus formation in a rabbit femoral artery eversion graft preparation in 4/5 animals (34). An active-site inactivated factor VIIa (DEGR-VIIa) or tissue factor pathway inhibitor (TFPI) inhibited angiographic restenosis and intimal hyperplasia in an atherosclerotic rabbit balloon injury model (35). Exogenously administered TFPI has been shown to inhibit venous thrombosis in rabbits (36) and to attenuate stenosis in balloon-injured hyperlipidemic pigs (37). Although these studies are small, they do point to a central role for TF in mediating thrombosis associated with acute arterial injury. In addition, they suggest that TF-mediated thrombosis may contribute to intimal hyperplasia and luminal narrowing.

Cellular regulation of TF

The above studies have implicated macrophages, SMC, and perhaps endothelial cells as sources of TF in the arterial wall. While the agents responsible for TF induction *in vivo* remain to be determined, considerable information exists as to the regulation of TF in cell culture.

In endothelial cell culture, TF mRNA and/or procoagulant activity is induced by phorbol esters (38,39), tumor necrosis factor (38,40,41), endotoxin (39,42), interleukin 1 (43) and a-thrombin (44). Similar results have been found in monocytes, where TF mRNA and/or procoagulant activity is stimulated by the above agents as well as a variety of mediators of inflammation and antigen-specific cellular immune responses (reviewed in 12). TF expression in endothelial cells is also regulated by shear stress (45). The regulation of TF gene expression in these cell types has been extensively studied and shown to involve cooperative activation of several elements on the human TF promoter, and in particular the NFkB binding site (reviewed in 46).

The presence of TF activity in cultured SMC was initially reported by Maynard and coworkers (24). We examined the regulation of TF gene expression in cultured rat aortic VSMC (47). TF mRNA and procoagulant activity were found to be rapidly and markedly induced (≈10-fold) in early- and late-passaged rat aortic VSMC by serum, PDGF, epidermal growth factor, angiotensin II, and a-thrombin (47). The induction of TF mRNA by these agents was dependent upon mobilization of intracellular Ca^{2+}, but not on changes in extracellular Ca^{2+}. Unlike other growth factor-responsive genes in SMC, down-regulation of protein kinase C activity by prolonged treatment with phorbol esters failed to block agonist-mediated TF induction. The induction of TF in SMC and endothelial cells by thrombin is of particular note because thrombin is generated from prothrombin as a consequence of activation of the TF-mediated coagulation cascade. Thus, a positive feedback mechanism exists whereby thrombin, generated by TF activation, can perpetuate a procoagulant state by inducing further TF synthesis.

We have recently extended cell culture studies to human aortic and coronary artery SMC. Serum, PDGF, and α-thrombin induced TF mRNA and protein in both cell types (48). By nuclear run-off analysis, PDGF-induced TF transcription peaked at 30 minutes. Of note, PDGF AA, which is induced in the vessel wall by injury, was a potent agonist for TF. By light and confocal microscopy, TF antigen was localized to the perinuclear cytoplasm beginning 2 hours after agonist treatment and persisting for 10-12 hours. TF antigen subsequently appeared as patches near the surface. Human SMC monolayers were assayed for surface activity in a parallel plate flow chamber, similar to that previously used for endothelial cells (45). TF activity on the cell surface peaked at 4-6 hours and returned to baseline within 16 hours. Surface TF activity accounted for <20% of the total activity measured in cell lysates. Thus in contrast to macrophages, where TF is predominantly localized on the surface, SMC accumulate TF within the cytoplasm. The transport of TF to the SMC surface may be a critical step in regulating SMC-mediated thrombosis in acute arterial injury.

In addition to its induction in SMC by growth agonists, TF mRNA and protein was also induced in SMC and in THP-1 myelomonocytic leukemia cells by monocyte chemoattractant protein (MCP) -1 (49). MCP-1 is a potent monocyte chemotactic factor that is found in abundance in atherosclerotic plaques (50,51). The induction of TF mRNA

and protein by MCP-1 occurred with a time course (1-6 hours) and with levels similar to those found in PDGF-stimulated SMC or LPS-stimulated THP-1 cells. Chelation of intracellular calcium and inhibition of protein kinase C blocked the induction of TF in SMC by MCP-1, suggesting that it is mediated by activation of phospholipase C. Because MCP-1 is likely involved in recruiting macrophages to atherosclerotic lesions and is present at high concentrations in macrophage-rich areas, it may serve as an important agonist for inducing TF in the macrophages of the atherosclerotic plaque. In addition, as one of the more abundant proteins secreted by SMC, it may also act in an autocrine fashion to induce TF in these cells, particularly in the setting of acute arterial injury.

Summary

TF antigen and activity are found in abundance in human atherosclerotic plaques, particularly in the lipid-rich core. TF is also readily induced in the arterial wall by balloon injury and accumulates in the resulting neointima. In chronic atherosclerosis, the macrophage is likely to be the major source of TF within the plaque. TF accumulates as an early event associated with the migration of monocytes to the vessel wall in response to chemoattractants, such as MCP-1, and their differentiation into macrophages. As SMC become activated in the developing plaque, they provide a second source of TF. Macrophages and SMC accumulate lipid and become foam cells, ultimately degenerating into a necrotic core rich in TF. Spontaneous plaque rupture or acute interventions expose active TF in the core to circulating blood, triggering thrombosis.

In acute arterial injury, SMC appear to be the chief source of TF. In normal vessels, the induction of TF in the medial SMC is not sufficient to generate fibrin, presumably because the TF is not readily accessible on the luminal surface. In contrast, endothelial denudation of previously injured arteries may expose intimal TF to circulating blood, resulting in rapid fibrin deposition. In advanced human atherosclerosis, it is likely that even in areas that do not contain "unstable" or "stable" plaques, the vessel wall is not normal and more closely resembles that of a previously injured artery possessing an active intima. Interventions, such as balloon angioplasty, coronary atherectomy, or stent placement may expose intimal TF, leading to fibrin deposition.

As the initiator of coagulation, TF is a potential target for inhibiting the thrombotic complications of atherosclerosis. TFPI (reviewed in 52) is currently under clinical investigation as an anticoagulant and its effects on intimal hyperplasia in animal models are being studied. Direct factor Xa inhibitors, such as tick anticoagulant peptide (TAP) and leech anticoagulant peptide (ATS), are also under investigation (53,54). Finally, the recent crystallization of TF (55) and the TF:VIIa (56) should provide important new insights into the design of molecules for directly inhibiting TF.

References

1. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992, 326:242-250.
2. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992, 326:310-318.
3. Losordo DW, Rosenfield K, Pieczek A, Baker K, Harding M, Isner JM. How does angioplasty work?: serial analysis of human iliac arteries using intravascular ultrasound. Circulation 1992, 86:1845-1858.
4. Nath FC, Muller DW, Ellis SG, Rosenschein U, Chapekis A, Quain L, Zimmerman C, Topol EJ. Thrombosis of a flexible coil coronary stent: frequency, predictors and clinical outcome. J Amer Col Card 1993, 21:622-627.
5. Carrozza Jr JP, Baim DS. Complications of directional coronary atherectomy: incidence, causes, and management. Am J Card 1993, 72:47E-54E.
6. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993, 362:801-809.
7. Schwartz SM, Heimark RL, Majesky MW. Developmental mechanisms underlying pathology of arteries. Physiol Rev 1990, 70:1177-1209.
8. Huang CL, Ives HE. Growth inhibition by protein kinase C late in mitogenesis. Nature 1987, 329:849-850.
9. Gasic GP, Arenas CP, Gasic TB, Gasic, GJ. Coagulation factors X, Xa, and protein S as potent mitogens of cultured aortic smooth muscle cells. Proc Natl Acad Sci, USA 1992, 89:2317-2320.
10. Ragosta M., Gimple LW, Haber HL, Dunwiddie CT, Vlasuk GP, Rowers ER, Sarembock IJ. Effectiveness of specific Factor Xa inhibition on restenosis following balloon angioplasty in rabbits. J Amer Col Cardiol 1992, 19:164A.
11. Nemerson Y. Tissue factor and hemostasis. Blood 1988, 71:1-8.
12. Edgington TS, Mackman N, Brand K, Ruf W. The structural biology of expression and function of tissue factor. Thromb Haemost 1991, 66:67-79.
13. Davies MJ, Thomas AC. Plaque fissuring- the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. Br Heart J 1985, 53:363-373.
14. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Am J Path 1989, 134:1087-1097.
15. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci, USA 1989, 86:2839-2843.
16. Annex BH, Denning SM, Channon KM, Sketch MH Jr, Stack RS, Morrissey JH, Peters KG. Differential expression of tissue factor protein in directional atherectomy specimens from patients with stable and unstable coronary syndromes. Circulation 1995, 91:619-622.
17. Marmur JD, Thiruvikraman SV, Fyfe BS, Guha A, Sharma SK, Ambrose JA, Fallon JT, Nemerson Y, Taubman MB. The identification of active tissue factor in human coronary atheroma. Circulation, September, 1996.
18. Moreno PR, Bernardi VH, Lopez-Cuellar J, Murcia AM, Palacios IF, Gold HK, Mehran R, Sharma SK, Nemerson Y, Fuster V, Fallon J. macrophages, smooth muscle cells, and tissue factor in unstable angina. Circulation 1996, 94:3090-3097.
19. Thiruvikraman SV, Guha A, Roboz J, Taubman MB, Nemerson Y, Fallon JT. In situ localization of tissue factor in human atherosclerotic plaques by binding of digoxigenin labeled factors VIIa and X.. Submitted, Lab Invest.
20. Badimon L, Badimon JJ, Galvez A, Chesebro J, Fuster V. Influence of arterial damage and wall shear rate on platelet deposition. Arteriosclerosis 1986, 6:312-320.
21. Mailhac A, Badimon JJ, Fallon JT, Fernandez-Ortiz A, Meyer B, Chesebro JH, Fuster V, Badimon L. Effect of an eccentric severe stenosis on fibrin(ogen) deposition on severely damaged vessel

wall in arterial thrombosis. Relative contribution of fibrin(ogen) and platelets. Circulation 1994, 90:988-996.
22. Fernandez-Ortiz A, Badimon JJ, Falk E, Fuster V, Meyer B, Mailhac A, Weng D, Shah PK, Badimon L. Characterization of the relative thrombogenicity of atherosclerotic plaque components: implications for consequences of plaque rupture. J Am Coll Cardiol 1994, 23:1562-1569.
23. Badimon JJ, Weng D, Chesebro JH, Fuster V, Badimon L. Platelet deposition induced by severely damaged vessel wall is inhibited by a boroarginine synthetic peptide with antithrombin activity. Thromb Haemost 1994, 71:511-516.
24. Maynard JR, Heckman CA, Pitlick FA, Nemerson Y. Association of tissue factor activity with the surface of cultured cells. J Clin Invest 1975, 55:814-824.
25. Steele PM, Chesebro JH, Stanson AW, Holmes D, Badimon L, Fuster V. Balloon angioplasty. Natural history of the pathophysiological response to injury in a pig model. Circ Res 1985, 57:105-112.
26. Stemerman MB. Thrombogenesis of the rabbit arterial plaque. An electron microscopic study. Am J Path 1973, 81:15-42.
27. Clowes AW, Reidy MA, Clowes MM. Kinetics of cellular proliferation after arterial injury. Lab Invest 1983, 49:327-333.
28. Richardson M, Kinlough-Rathbone RL, Groves HM, Jorgensen J, Mustard JF, Moore S. Ultrastructural changes in re-endothelialized and non-endothelialized rabbit aortic neo-intima following re-injury with a balloon catheter. Br J Exp Path 1984, 64:597-611.
29. Groves HM, Kinlough-Rathbone RL, Richardson M, Jorgensen J, Moore S, Mustard JF. Thrombin generation and fibrin formation following injury to rabbit neointima. Studies of vessel wall reactivity and platelet survival. Lab Invest 1982, 46:605-612.
30. Marmur JD, Rossikhina M, Guha A, Fyfe B, Friedrich V, Mendlowitz M, Nemerson Y, Taubman MB. Tissue factor is rapidly induced in arterial smooth muscle after balloon injury. J Clin Invest 1993, 91:2253-2259.
31. Pawashe AB, Golino P, Ambrosis G, Migliaccio F, Ragni M, Pascucci I, Chiariello M, Bach R, Garen A, Konigsberg WK, Ezekowitz MD. A monoclonal antibody against rabbit tissue factor inhibits thrombus formation in stenotic injured rabbit carotid arteries. Circ Res 1994, 74:56-63.
32. Speidel CM, Eisenberg PR, Ruf W, Edgington TS, Abendschein DR. Tissue factor mediates prolonged procoagulant activity on the luminal surface of balloon-injured aortas in rabbits. Circulation 1995, 92:3323-3330.
33. Gallo R, Fallon JT, Toschi V, Gertz SD, Padurean A, Nemerson Y, Chesebro J, Fuster V, Badimon JJ. Bi-phasic increase of tissue factor activity after angioplasty in porcine coronary arteries. Circulation 1995, 92:I-354.
34. Jang IK, Gold HK, Leinbach RC, Fallon JT, Collen D, and Wilcox JN. Antithrombotic effect of a monoclonal antibody against tissue factor in a rabbit model of platelet-mediated arterial thrombosis. Arterioscler Thromb 1992, 12:948-954.
35. Jang Y, Guzman LA, Lincoff AM, Gottsauner-Wolf M, Forudi F, Hart CE, Courtman DW, Ezban M, Ellis SG, Topol EJ. Influence of blockade at specific levels of the coagulation cascade on restenosis in a rabbit atherosclerotic femoral artery injury model. Circulation 1995, 92:3041-3050.
36. Holst J, Lindblad B, Bergqvist D, Nordfang O, Ostergaard PB, Petersen JG, Nielsen G, Hedner U. Antithrombotic effect of recombinant truncated tissue factor pathway inhibitor (TFPI1-161) in experimental venous thrombosis - a comparison with low molecular weight herpatin. Thromb Haemost 1994, 71:214-219.
37. Oltrana L, Speidel CM, Recchia D, Abendschein DR. Inhibition of tissue factor-mediated thrombosis markedly attenuates stenosis after balloon-induced arterial injury in hyperlipidemic minipigs. Circulation 1994, 90:I-344.
38. Scarpati EM, Sadler JE. Regulation of endothelial cell coagulant properties. J Biol Chem 1989, 264:20705-20713.
39. Crossman DC, Carr DP, Tuddenham EGD, Pearson JD, McVey JH. Regulation of tissue factor mRNA in human endothelial cells in response to endotoxin or phorbol ester J Biol Chem 1990, 265:9782-9787.
40. Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 1986, 163:740-745.
41. Conway EM, Bach R, Rosenberg RD, Konigsberg WH. Tumor necrosis factor enhances expression of tissue factor mRNA in endothelial cells. Thromb Res 1989, 53:231-241.
42. Moore KL, Andreoli SP, Esmon NL, Esmon CT, Bang NU. Endotoxin enhances tissue factor and suppresses thrombomodulin expression of human vascular endothelium in vitro. J Clin Invest 1987, 79:124-130.
43. Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone Jr MA. Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. J Exp Med 1984, 160:618-623.
44. Brox JH, Osterud B, Bjrklid E, Fenton II JW. Production and availability of thromboplastin in endothelial cells: the effects of thrombin, endotoxin and platelets. Br J Haematol 1984, 57:239-246.
45. Grabowski EF, Lam FP. Endothelial cell function, including tissue factor expression, under flow conditions. Thromb Haemost 1995, 74:123-128.
46. Mackman N. Regulation of the tissue factor gene. FASEB J 1995, 9:883-889.
47. Taubman MB, Marmur JD, Rosenfield CL, Guha A, Nichtberger S, Nemerson Y. Agonist-mediated tissue factor expression in cultured vascular smooth muscle cells: Role of Ca^{2+} mobilization and protein kinase C activation. J Clin Invest 1993, 91:547-552.
48. Schecter AD, Fallon JT, Thiruvikraman SV, Taby O, Rosenfield CL, Rossikhina M, Nemerson Y, Taubman MB. Delayed surface expression of active tissue factor in human arterial smooth muscle cells determined by a novel in situ activity assay. Circulation 92:I-804.
49. Schecter AD, Fallon JT, Rossikhina M, Xhang X, Nemerson Y, Taubman MB. Monocyte chemoattractant protein 1 (MCP-1) induces tissue factor in human arterial smooth msucle cells (SMC) and monocytes. Blood 1995, 86:286a.
50. Yla-Herttuala S, Lipton BA, Rosenfeld ME, Sarkioja T, Yoshimura T, Leonard EJ, Witztum JL, Steinberg D. Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. Proc Natl Acad Sci USA 1991, 88:5252-5256.
51. Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 1991, 88:1121-1127.
52. Broze Jr GJ. Tissue factor pathway inhibitor. Thromb Haemost 1995, 74:90-93.
53. Waxman L, Smith DE, Arcuri KE, Vlasuk GP. Tick anticoagulant peptide (TAP) is a novel inhibitor of blood coagulation factor Xa, Science 1990, 248:593-596.
54. Dunwiddie CT, Thornberry N, Bull H, Sardana M, Friedman P, Jacobs J, Simpson E. Antistasin, a leech-derived inhibitor of factor Xa: Kinetic analysis of enayme inhibition and identification of the reactive site. J Biol Chem 1989, 264:16694-16699.
55. Harlos K, Martin DM, O'Brien DP, Jones EY, Stuart DI, Polikarpov I, Miller A, Tuddenham EG, Boys CW. Crystal structure of the extracellular region of human tissue factor. Nature 1994, 370:662-666.
56. Banner DW, D'Arcy A, Chene C, Winkler FK, Guha A, Konigsberg WH, Nemerson Y, Kirchhofer D. The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. Nature 1996, 380:41-46.
57. Zeldis S, Nemerson Y, Pitlick R, Lentz T. Tissue factor (thromboplastin): localization by peroxidase conjugated antibodies. Science 1972, 175:766-768.