Published in final edited form as:

Thromb Res. 2012 May ; 129(Suppl 2): S21–S22. doi:10.1016/j.thromres.2012.02.023.

Platelet Tissue Factor Pathway Inhibitor Modulates Intravascular Coagulation

Susan A. Maroney and Alan E. Mast

Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI

Abstract

Tissue factor pathway inhibitor (TFPI) is produced by megakaryocytes and is found internally within quiescent platelets but is not in α -granules. It is released in soluble form and expressed on the surface of platelets that are dual activated with thrombin plus collagen. Platelet TFPI is exclusively TFPI α , the most evolutionarily conserved TFPI isoform. It appears to be physiologically active as an inhibitor of tissue factor (TF) initiated FXa generation *in vitro*, and acts locally to dampen clot growth in a murine vascular injury model. These data suggest that platelet TFPI plays an important role in modulating TF activity within a growing clot thereby preventing formation of an occlusive clot.

Introduction

Clot formation is initiated following vascular injury when tissue factor of the subendothelium comes in contact with blood FVIIa forming the TF/FVIIa complex that activates FX. Both the TF/FVIIa complex and FXa of this clot initiation phase are inhibited by tissue factor pathway inhibitor (TFPI). TFPI is a protease inhibitor found in endothelial cells [1], platelets [2,3], plasma [4,5], monocytes [6] and a variety of other cells [7]. The majority of TFPI is located in the endothelial cells (85%) [8], while the platelets account for 7–10% of that found within whole blood [2,3]. Since platelets accumulate at sites of vascular injury, TFPI within platelets may have an important anticoagulant function in balancing adequate hemostasis without excessive clot formation.

TFPI is made in at least two alternatively spliced isoforms, TFPI α and TFPI β [9,10]. TFPI α consists of a signal peptide followed by three Kunitz domains with stretches of amino acids between the domains. The TFPI first Kunitz domain binds the TF/FVIIa complex and the second Kunitz domain binds FXa resulting in the formation of a ternary complex of TF/FVIIa/FXa/TFPI [11]. The third Kunitz domain binds to protein S, which enhances the FXa inhibitory activity of soluble TFPI α [12,13]. Following the third Kunitz domain, a highly basic C-terminal tail allows binding of TFPI to the endothelial surface glycosaminoglycans (GAG). In humans, this binding is interrupted following heparin administration allowing TFPI to be released and increases the TFPI plasma concentrations 2 to 4-fold [5,14]. TFPI β consists of the signal peptide and the first two Kunitz domains. It has a unique 8–12 amino

© 2012 Elsevier Ltd. All rights reserved.

Corresponding Author: Susan A. Maroney, Blood Research Institute, 8727 Watertown Plank Road, PO Box 2178, Milwaukee, WI 53201-2178, Tel: 414-937-3884, Fax: 414-937-6284, susan.maroney@bcw.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

acid sequence at its C-terminus that is attached to the cell surface via a glycosylphosphatidylinositol (GPI)-anchor [9].

Of particular interest is that human and mouse platelets make exclusively TFPI α [15]. This contrasts with adult mouse tissue vascular beds [16] and cultured human endothelial cells (Girard TJ et al., Blood In Press), which express the TFPI β isoform. TFPI α is evolutionarily conserved back to zebrafish, 400 million years, while TFPI β is found only in mammals and is, therefore, more recently evolved [16]. Just as platelets have evolved from *Limulus* amebocytes, 400 million years, and still retain many of the properties of these single circulating cells with multiple functions, one of which is hemostasis [17]; the expression of the evolutionarily conserved TFPI α isoform in platelets suggests that it retains an important biological function.

TFPI α is synthesized by megakaryocytes

TFPI α is present within both mouse and human platelets [3]. Western blot analysis using antibodies specific for the C-terminal region of TFPI α or deglycosylation to separate TFPI α and TFPI β have demonstrated TFPI α as the only alternatively spliced TFPI isoform present within human and mouse platelets. Mouse megakaryocyte cultures were utilized to determine if TFPI synthesized by megakaryocytes or adsorbed from plasma by platelets. Mouse bone marrow cultures differentiated into megakaryocytes were permeabilized, fixed and stained for mouse TFPI. Using confocal microscopy, it was determined that TFPI was found within the mouse megakaryocytes, but not on their surface. The first evidence that TFPI mRNA was found within platelets was using Northern blot analysis that determined human platelets contain the entire protein coding region of TFPI [2]. Later, quantitative RT-PCR for both TFPI α and TFPI β was performed on highly purified platelets and found that TFPI α message was present in the purified platelet preparation while TFPI β was not present [3]. Collectively, these data indicate that TFPI α is the isoform exclusively made by both mouse and human platelets.

At this time, it is unknown where TFPI is stored within platelets. Human and mouse platelets or megakaryocytes were used to detect the location of TFPI within platelet structures. TFPI did not co-localize with von Willebrand factor (VWF), fibrinogen, or lysosomal-associated membrane protein-1 (LAMP-1) [3]. Therefore, TFPI is thought to localize to a platelet compartment other than α -granules or in lysosomes.

TFPI is expressed on the surface of coated platelets

Platelet TFPI was first described in 1988 when it was demonstrated that stimulation of platelets with thrombin resulted in secretion of soluble TFPI. TFPI was not expressed on the platelet surface or shed in vesicles [2]. Evidence of the release of platelet TFPI at the site of a wound was demonstrated by measurement of TFPI in blood samples obtained from a skin wound. Over time, the TFPI concentration in the oozing blood increased 2 to 3-fold over the initial plasma concentration. Interestingly, the increase in TFPI lagged the increase in platelet factor 4 suggesting a delayed release of TFPI from a platelet compartment other than α -granules [2].

Our laboratory discovered that TFPI is expressed on the surface of platelets following activation with thrombin in conjunction with either convulxin or calcium ionophore [3]. Platelets activated with the combination of thrombin and convulxin produced TFPI surface expression on 35–50% of the platelets. The percentage of platelets expressing TFPI on the surface increased to greater than 95% when the combination of thrombin and calcium ionophore was used to stimulate the platelets. TFPI is expressed on the surface of less than 1% of quiescent platelets, platelets stimulated using high levels of thrombin receptor agonist

peptide (TRAP), or other single agonists as determined by flow cytometry [3]. Therefore, it appears that dual agonist activation is necessary for expression of TFPI on the surface of platelets.

Differential centrifugation was used to detect the location of membrane bound TFPI in quiescent and activated platelets. In quiescent platelets, TFPI was located within intact platelets but not within microvesicles membranes, exosome membranes, or as a soluble protein. However, when platelets were stimulated with dual agonists thrombin and calcium ionophore for 2 minutes, TFPI was located on intact platelet membranes, microvesicle membranes, and as a soluble protein [3]. In addition, the total TFPI (surface expressed and secreted) inhibited tissue factor (TF) activity in a TF/FVIIa initiated FXa generation activity assay that was reversible with anti-TFPI antibody [3]. These findings of TFPI platelet surface expression and release from dual agonist stimulated platelets that functionally inhibits TF activity suggest that platelet TFPI acts physiologically to prevent intravascular coagulation, hence further expansion of vascular plug thus maintain vascular patency.

Platelet TFPI acts locally to limit clot formation

Microparticle-associated TF has been shown to improve clot formation in a hemophilia mouse model [18]. To test the hypothesis that platelet TFPI locally regulates TF initiated clot formation, fetal liver transplantation was used to generate mice lacking TFPI in hematopoietic cells. In this model, $Tfpi^{+/-}$ mice, possessing TFPI plasma at a concentration of approximately 50% of that found in $Tfpi^{+/+}$ mice (24 nM vs. 44 nM), were transplanted with hematopoietic precursor cells of either the $Tfpi^{+/+}$ or $Tfpi^{-/-}$ genotype [15]. Following engraftment of the transplanted cells, the mice demonstrated no differences in their hematocrit, white blood cell count, platelet count, plasma TFPI concentration, or plasma thrombin-anti-thrombin (TAT) concentration, indicating that the lack of hematopoietic cells TFPI does not produce a global procoagulant state. These mice were evaluated in a venous electrolytic injury model. Mice transplanted with $Tfpi^{-/-}$ hematopoietic cells produced larger clots than did the mice transplanted with $Tfpi^{+/+}$ hematopoietic cells. The clot profile of these mice was essentially identical within the first 12 minutes of the clot time. However, platelet accumulation continued in the mice transplanted with $Tfpi^{-/-}$ hematopoietic cells while the mice transplanted with $Tfpi^{+/+}$ hematopoietic cells began to decrease after 14 minutes of clot initiation. At 30 minutes, mice transplanted with $Tfpi^{-/-}$ hematopoietic cells had significantly more platelets in the thrombus over that of the mice transplanted with $Tfpi^{+/+}$ hematopoietic cells. These data demonstrate that hematopoietic TFPI, most likely platelet TFPI, limited the size of the clot by limiting platelet accumulation secondary to blood-borne TF accumulation.

Summary

Both human and mouse platelets contain $TFPI\alpha$, which is the most evolutionarily conserved alternatively spliced form of TFPI present in zebra fish up through humans. TFPI localizes within quiescent platelets but is not present in α -granules or lysosomes. As such, it is not as easily expressed on the platelet surface, requiring dual agonist stimulation with collagen plus thrombin. Along with its surface expression, release of TFPI in soluble form occurs during stimulation of platelets with either thrombin or with dual stimulation of collagen and thrombin. Platelet TFPI is expressed on the platelet surface and released from activated platelets accumulating at the site of vascular injury where it acts physiologically to inhibit the TF/FVIIa procoagulant complex and dampen intravascular thrombus growth.

References

1. Bajaj MS, Kuppuswamy MN, Saito H, Spitzer SG, Bajaj SP. Cultured Normal Human Hepatocytes do not Synthesize Lipoprotein-Associated Coagulation Inhibitor: Evidence that Endothelium is the Principal Site of Its Synthesis. *PNAS*. 1990; 87:8869–8873. [PubMed: 2247459]
2. Novotny WF, Girard TJ, Miletich JP, Broze GJ Jr. Platelets secrete a coagulation inhibitor functionally and antigenically similar to the lipoprotein associated coagulation inhibitor. *Blood*. 1988; 72:2020–2025. [PubMed: 3143429]
3. Maroney SA, Haberichter SL, Friesse P, Collins ML, Ferrel JP, Dale GL, Mast AE. Active tissue factor pathway inhibitor is expressed on the surface of coated platelets. *Blood*. 2007; 109:1931–1937. [PubMed: 17082321]
4. Novotny WF, Girard TJ, Miletich JP, Broze GJ Jr. Purification and characterization of the lipoprotein-associated coagulation inhibitor from human plasma. *J Biol Chem*. 1989; 264:18832–18837. [PubMed: 2553722]
5. Sandset PM, Abildgaard U, Larsen ML. Heparin induces release of extrinsic coagulation pathway inhibitor (EPI). *Thromb Res*. 1988; 50:803–813. [PubMed: 3413731]
6. Ott I, Andrassy M, Zieglansberger D, Geith S, Schomig A, Neumann FJ. Regulation of monocyte procoagulant activity in acute myocardial infarction: role of tissue factor and tissue factor pathway inhibitor-1. *Blood*. 2001; 97:3721–3726. [PubMed: 11389008]
7. Bajaj MS, Birktoft JJ, Steer SA, Bajaj SP. Structure and biology of tissue factor pathway inhibitor. *Thromb Haemost*. 2001; 86:959–972. [PubMed: 11686353]
8. Werling RW, Zacharski LR, Kisiel W, Bajaj SP, Memoli VA, Rousseau SM. Distribution of tissue factor pathway inhibitor in normal and malignant human tissues. *Thromb Haemost*. 1993; 69:366–369. [PubMed: 8497849]
9. Chang JY, Monroe DM, Oliver JA, Roberts HR. TFPIbeta, a second product from the mouse tissue factor pathway inhibitor (TFPI) gene. *Thromb Haemost*. 1999; 81:45–49. [PubMed: 9974373]
10. Maroney SA, Ferrel JP, Collins ML, Mast AE. TFPIgamma is an active alternatively spliced form of TFPI present in mice but not in humans. *J Thromb Haemost*. 2008
11. Girard TJ, Warren LA, Novotny WF, Likert KM, Brown SG, Miletich JP, Broze GJ Jr. Functional significance of the Kunitz-type inhibitory domains of lipoprotein-associated coagulation inhibitor. *Nature*. 1989; 338:518–520. [PubMed: 2927510]
12. Hackeng TM, Sere KM, Tans G, Rosing J. Protein S stimulates inhibition of the tissue factor pathway by tissue factor pathway inhibitor. *Proc Natl Acad Sci U S A*. 2006; 103:3106–3111. [PubMed: 16488980]
13. Ndonwi M, Tuley EA, Broze GJ Jr. The Kunitz-3 domain of TFPI-alpha is required for protein S-dependent enhancement of factor Xa inhibition. *Blood*. 2010; 116:1344–1351. [PubMed: 20479289]
14. Novotny WF, Palmier M, Wun TC, Broze GJ Jr, Miletich JP. Purification and properties of heparin-releasable lipoprotein-associated coagulation inhibitor. *Blood*. 1991; 78:394–400. [PubMed: 2070077]
15. Maroney SA, Cooley BC, Ferrel JP, Bonesho CE, Mast AE. Murine hematopoietic cell tissue factor pathway inhibitor limits thrombus growth. *Arterioscler Thromb Vasc Biol*. 2011; 31:821–826. [PubMed: 21233452]
16. Maroney SA, Ferrel JP, Pan S, White TA, Simari RD, McVey JH, Mast AE. Temporal expression of alternatively spliced forms of tissue factor pathway inhibitor in mice. *J Thromb Haemost*. 2009; 7:1106–1113. [PubMed: 19422457]
17. Levin, J. The evolution of mammalian platelets. In: Michelson, AD., editor. *Platelets*. NY: Academic Press; 2002. p. 3–14.
18. Hrachovinova I, Cambien B, Hafezi-Moghadam A, Kappelmayer J, Camphausen RT, Widom A, Xia L, Kazazian HH, Schaub RG, McEver RP, Wagner DD. Interaction of P-selectin and PSGL-1 generates microparticles that correct hemostasis in a mouse model of hemophilia A. *Nat Med*. 2003; 9:1020–1025. [PubMed: 12858167]