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Interdependent biological systems, multi-functional molecules: the evolving role of tissue factor pathway inhibitor beyond anti-coagulation

Eric Holroyd, M.B., B.Chir., MRCP. and Robert D. Simari, M.D.

Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota, 55905

Abstract

Coagulation, innate immunity, angiogenesis, and lipid metabolism represent fundamental and interdependent biological systems. While tissue factor pathway inhibitor (TFPI) is the major physiological inhibitor of TF, its unique structure and endothelial expression allow multi-modal interactions with constituent molecules in each of these systems. We review emerging data describing roles for TFPI beyond simply opposing the action of TF, particularly with regard to the highly basic c-terminus of TFPI, and highlight potentially exciting new areas for future research.

Keywords

Tissue factor pathway inhibitor; tissue factor; inflammation; angiogenesis; lipid metabolism; atherosclerosis

Introduction

The biological systems of coagulation, inflammation, angiogenesis, and lipid metabolism show considerable interdependence and functional overlap. Cloning data and comparative sequence analysis indicate that the entire coagulation system is present in all jawed vertebrates and probably evolved prior to the divergence of jawless fish 450 million years ago [1]. From zebra fish to *homo sapiens*, highly conserved, multi-functional molecules perform their vital functions in a variety of systems. Tissue factor (TF) and now tissue factor pathway inhibitor (TFPI) have emerged as integral components to these phylogenetically ancient systems.

TFPI is the major physiological inhibitor of TF. It is a multivalent serine protease inhibitor with 3 independently folded Kunitz-type protease inhibitor domains [2] and a highly basic c-terminus, present on the endothelial cell surface. The first Kunitz domain binds TF/VIIa complex [3], the second binds factor Xa. It is the formation of this quaternary TF-VIIa-TFPI-Xa complex that constitutes the ‘classical’ role of TFPI and dampens ongoing coagulation.

Address for correspondence: Robert D. Simari, M.D., Mayo Clinic, Division of Cardiovascular Diseases, 200 First Street SW, Rochester, Minnesota, 55905, USA, Fax: 507-538-6418, Phone: 507-284-3727, simari.robert@mayo.edu.

Conflict of interest statement

There are no conflicts of interests to disclose.

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New roles for TFPI have been identified in inflammation, angiogenesis, and lipid metabolism, beyond simply opposing the action of TF.

Innate immunity

Inflammation is a local response to cellular injury. It is an important part of the innate host immune mechanism. Pro-inflammatory molecules released by injured cells lead to the classical symptoms of *rubor* (redness), *calor* (heat), *tumor* (swelling), and *dolor* (pain), the establishment of a physical barrier to infection and promotion of healing. Overwhelming sepsis may trigger excessive activation of pro-inflammatory cytokines and a systemic, rather than local, inflammatory response leading to hypotension, disseminated intravascular coagulopathy (DIC), multi-organ failure, and ultimately death.

Inflammation-induced coagulation is a well recognized phenomenon [4], many primarily inflammatory cytokines (such as IL-1 β , IL-6, TNF α) can trigger the coagulation cascade either directly or indirectly by up-regulating pro-coagulant factors in vascular cells (such as TF). Coagulation-induced inflammation, however, is a more novel concept [5]. TF, thrombin, factor Xa can all induce inflammation. Indeed, TF can play a central role in systemic inflammatory conditions, such as Gram-negative sepsis and inhibition of TF signaling may offer a potential therapeutic target.

TF, a transmembrane glycoprotein present on the surface of most extravascular cells, is the primary cellular initiator of coagulation. Inflammatory cytokines (TNF α , IL-1) can stimulate expression of TF by endothelial cells [6–8]. TF classically triggers coagulation in complex with factor VIIa (TF-VIIa). This same molecular complex has potent signaling ability in numerous other systems and cells. TF-VIIa cleaves and activates protease activated receptor 2 (PAR2) on the cell surface leading to the production of pro-inflammatory cytokines and proteins (including IL-1, IL-6 and IL-8)[9,10]. In vivo models of Gram negative sepsis confirm the role of TF-VIIa signaling and an inhibitory, modulatory role for TFPI. Genetically modified mice expressing low levels of TF in all tissues or hematopoietic tissue-specific knock out of TF had reduced coagulation, inflammation (less IL-6 and TNF α), and mortality following intraperitoneal lipopolysaccharide (LPS) injection [11]. Baboons pretreated with anti-TF antibodies show reduced coagulopathy and mortality with an *E. coli* sepsis model [12]. Similarly, TFPI has been shown in animal models to attenuate inflammation and coagulopathy during sepsis. TFPI treated mice were protected in an intraabdominal sepsis induction model, showing reduced plasma IL-6 levels and improved survival [13]. Baboons receiving lethal doses of *E. coli* showed less hypotension, less inflammation (reduced plasma IL-6), and reduced mortality if given prior TFPI [14]. Unfortunately, human phase III trials of tifacogin, a synthetic TFPI analogue, failed to show a mortality benefit in critically ill sepsis patients [15].

Interestingly, recent evidence suggests TFPI could play a further more direct and independent role, beyond simply opposing the action of TF. TFPI contains a thrombin cleavage site that releases a 22 amino acid peptide [16]. Schirm et al [17] demonstrated that recombinant TFPI subject to proteolytic digestion (cathepsin G), but not full length TFPI or the proteases alone, suppressed bacterial growth in ex vivo whole blood cultures. This activity was localized to the c-terminal fragments of TFPI (TFPIct) which augmented complement mediated antibacterial activity. It may be that part of the benefit seen in earlier animal models of systemic sepsis was due to post-translational cleavage of TFPI and the opsonizing, antibacterial action of the TFPIct.

Angiogenesis

Angiogenesis is a fundamental biological process whereby hypoxia drives new blood vessel formation under the guidance of a milieu of pro- and anti-angiogenic factors. TF-VIIa can promote tumor growth and angiogenesis [18,19]. Elevated levels of TF correlate with an invasive carcinoma phenotype [20]. TF-VIIa promotes angiogenesis through PAR-2 signaling [21–23]. Adenoviral transfected endothelial cells expressing PAR2 and TF demonstrate reduced PAR2-signaling in the presence of recombinant TFPI [24]. The concentration of exogenous TFPI required to inhibit TF/PAR signaling in Chinese Hamster Ovary (CHO) cells is higher than that required to inhibit the coagulation cascade by TF/VIIa-dependent Xa generation, indicating distinct functional roles at different concentrations. The role of TFPI in regulating TF-VIIa/PAR2 signaling in vivo or in native cells, however, remains unknown. Furthermore, there is evidence to suggest that TFPI may not just oppose TF but act, via an as yet unknown mechanism, to independently inhibit angiogenesis [25,26].

TFPI exerts anti-tumor effects. Direct injection of TFPI around B16 melanoma tumors inhibits growth [25]. Although TFPI did not affect in vitro proliferation of B16 cells [26], it did inhibit proliferation of endothelial cells indicating that TFPI may act indirectly on tumor growth by inhibiting angiogenesis. Later studies demonstrated [27], using a TFPI c-terminal peptide (TFPIC23), inhibition of endothelial cell proliferation and increased apoptosis, both in the absence of TF, albeit at supraphysiological micromolar concentrations. Anti-tumor activity of TFPIC23 was also shown in an in vivo model.

The physiological concentration of TFPI in plasma is between 2.5 and 5 nM [28]. Provencal et al [29] highlighted the importance of using the appropriate concentration, showing that exogenous TFPI inhibits endothelial cell migration and tube formation at more physiological concentration (20nM) in vitro but had no effect on proliferation or evidence of cytotoxicity at this physiological concentration. TFPI was shown to inhibit ERK-1/2, FAK, and PAX phosphorylation in response to sphingosine-1-phosphate (S1P) stimulation. Importantly, this subtle action of TFPI, at physiological concentration, was demonstrated in the absence of TF. It is currently unknown how excess exogenous TFPI inhibits intracellular signaling pathways to inhibit migration and angiogenesis.

Lipid metabolism

Lipid metabolism involves the creation, trafficking, and degradation of lipids. Perhaps the paradigm interaction of lipid metabolism with other biological systems would be during the pathological process of atherosclerosis. Oxidized lipids taken up by dysfunctional endothelial cells stimulate inflammatory cells and cytokines to produce atherosclerotic plaques; intraplaque angiogenesis leads to instability, rupture, and thrombosis [30,31]. TFPI was originally named lipoprotein associated coagulation inhibitor (LACI) due to its binding of lipoproteins [32]. A new role has recently emerged for TFPI in lipoprotein and lipid metabolism which offers some of the clearest evidence yet of this multi-functional molecule acting in an independent, regulatory capacity.

TFPI α circulates in plasma predominantly bound to lipoproteins [33]. Plasma TFPI is cleared by binding heparin sulfate proteoglycans (HSPGs) and low density lipoprotein receptor related protein (LRP) [34,35]. TFPI requires its c-terminus to bind lipoproteins [36,37] and several cell surface receptors including the VLDL receptor [27]. Following vascular injury, vascular overexpression of TFPI in mice has been shown to reduce acute thrombosis and neointimal formation [38,39]. Conversely, TFPI haploinsufficiency increases the thrombotic tendency and atherosclerotic burden in pro-atherosclerotic Apo E deficient mice [40,41]. Interestingly, recent studies again involving mice overexpressing vascular TFPI bred into an Apo E deficient,

atherosclerotic, background not only revealed reduced plaque burden but also a reduction in total plasma cholesterol [42]. This suggested a role for TFPI in systemic lipid metabolism.

Further in vitro work revealed that TFPIct peptide stimulated a 15- to 20-fold increase in VLDL uptake, internalization, and degradation by murine embryonic fibroblasts (MEFs). Knockdown of the VLDL receptor using siRNA, only partially attenuated TFPIct stimulated VLDL binding and uptake, whereas prior heparanase treatment blocked TFPIct induced lipoprotein uptake. It appears TFPIct enhances binding and uptake of VLDL via a HSPG pathway. TFPIct is positively charged and was shown to associate with VLDL and affect its electrophoretic mobility. Indeed, intravenous injection of TFPIct alone was sufficient to significantly lower plasma cholesterol in Apo E deficient mice. Therefore, in this model system, TFPI, via its c-terminus, reduces atherosclerotic plaque formation by association with lipoproteins and stimulation of HSPG-dependent cellular uptake, lowering overall cholesterol levels.

Conclusion

Angiogenesis, coagulation, inflammation, and lipid metabolism are interdependent and fundamental biological processes that are remarkably conserved through almost half a billion years of evolution. The multi-functional nature of the constituent molecules in these systems is becoming increasingly clear. This allows an organism precise control over potentially dangerous interacting processes via complex feedback mechanisms; ensuring beneficial local processes, such as thrombosis or inflammation, do not become harmful and systemic, such as in DIC. TF and now TFPI can be considered integral components to all four systems.

Considering the paradigm of atherosclerosis, TFPI is ideally placed through its multimodal interactions, not only to antagonize the action of TF but also directly via its c-terminus, to act as an anti-inflammatory, anti-angiogenic, anti-coagulant, lipid lowering molecule. Further investigation of the multifunctional biology of TFPI will reveal much about pathophysiology of not only atherosclerosis but also the coordinate regulation of the fundamental biological systems that underlie the process and offer novel opportunities for therapeutic intervention.

Abbreviations

TFPI	Tissue factor pathway inhibitor
TF	tissue factor
DIC	disseminated intravascular coagulopathy
PAR2	protease activated receptor 2
CHO	Chinese hamster ovary
TFPIct	TFPI c-terminal fragments
HSPGs	heparin sulfate proteoglycans
LRP	lipoprotein receptor related protein
VLDL	very low density lipoprotein

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