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Topical Review

Endogenous Anticoagulants

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A B S T R A C T

Blood coagulation is a complex and highly coordinated process that is constantly altered and impacted by procoagulant and anticoagulant "players." It is vital that these components work in concert to maintain a balance to keep coagulation in check. Several important endogenous anticoagulants will be discussed in this review including tissue factor pathway inhibitor, antithrombin, protein C, and protein S in origin, structure, mechanism of action, effects of deficiency, and current knowledge in veterinary medicine.

Blood coagulation is a complex, highly coordinated process that is continuously modulated by procoagulant and anticoagulant "players." It is vital that these components work in concert to maintain a balance to keep coagulation in check. In certain disease states, particularly those with a massive inflammatory response, the scales are tipped toward a procoagulant state and the production of anticoagulant mediators is downregulated. The goal of this review is to discuss several important endogenous anticoagulants including tissue factor pathway inhibitor (TFPI), antithrombin (AT), protein C (PC), and protein S (PS) in origin, structure, mechanism of action, effects of deficiency, and current knowledge and applicability in veterinary medicine.

Tissue Factor Pathway Inhibitor

Background

Tissue factor (TF) is a transmembrane protein that acts as a receptor for plasma factor VII (FVII) and its activated form, FVIIa. Perivascular cells constitutively express TF, underlying the importance of its location to provide rapid activation of coagulation after vascular injury. TF is also constitutively expressed in the heart, kidney, brain, lungs, and placenta; these tissue-specific locations impart hemostatic protection in these highly vascular and vital organs.[1] When a vascular injury occurs, the adventitial cells expressing TF are exposed, allowing circulating FVIIa to bind to the uncovered TF. The TF-FVIIa complex then activates, through a positive feedback mechanism, FVII to FVIIa. Additionally, the TF-FVIIa complex activates small amounts of FIX and FX. This is the initiation step in the cell-based model of coagulation and acts as the primary initiator of coagulation in vivo.[2] More information regarding the cell-based model of coagulation can be found in the article in this issue. TFPI has a dual inhibitory function: it is the primary inhibitor of the TF-VIIa complex as well as an inhibitor of FXa.[1,2] Coagulation must be initiated for TFPI to function.[3]

Location

TFPI is primarily produced and expressed on the luminal surface of endothelial cells,[1,4] although megakaryocytes, monocytes, lung fibroblasts, and synovial cells are able to express low levels of TFPI. Approximately 10% of TFPI is bound to plasma lipoproteins[1,5,6] and a very small proportion circulates freely.[7] Activated platelets and heparin lead to the release of TFPI from intracellular stores.[8] Estimations of endogenous TFPI levels have been based on the amount of TFPI released after heparin injection.[7]

Structure and Mechanism of Action

TFPI is a Kunitz-type protease inhibitor containing Kunitz domains (K1-3) that inhibit the function of protein-degrading enzymes. In a 2-stage process, the second Kunitz domain, K2, binds to and directly inhibits FXa. The first domain, K1, then binds to a TF-FVIIa complex and inhibits it and prevents any further activation of FX. The formation of this quaternary structure is essential to the inhibitory actions of TFPI on TF-FVIIa. Although K3 is involved in lipoprotein binding and contains a heparin-binding site, it does not seem to function as a protease inhibitor[1,6] but is essential for optimal FXa inhibition. The specific role of K3 in the inhibition of coagulation appears to be through its relationship with protein S[4] (Fig. 1). The binding of TFPI to FXa is reversible and independent of calcium, whereas the binding of the TFPI-FXa complex to TF-VIIa is irreversible and requires calcium. Inactivation of TF-FVIIa by TFPI is both calcium- and FXa-dependent.[9] It should be noted that TFPI does not stop coagulation, but it limits further generation of FIXa and FXa by the TF-FVIIa complex.[10] TFPI can also stimulate monocytes to internalize and degrade TF-FVIIa complexes expressed on the cell surface.[11]

As stated earlier, heparin, both unfractionated and low molecular weight,[12] increases the levels of TFPI by inducing synthesis and causing secretion of TFPI by endothelial cells as well as displacing the bound portion[11,13] The inhibitory effects of TFPI are significantly enhanced in the presence of heparin.[14] As heparin is cleared from circulation, the effects of the heparin on TFPI cease and TFPI becomes endothelial bound again.[15]

Measurement of TFPI

TFPI can be measured by commercially available enzyme-linked immunosorbent assays (ELISA) and functional endpoint assays. The

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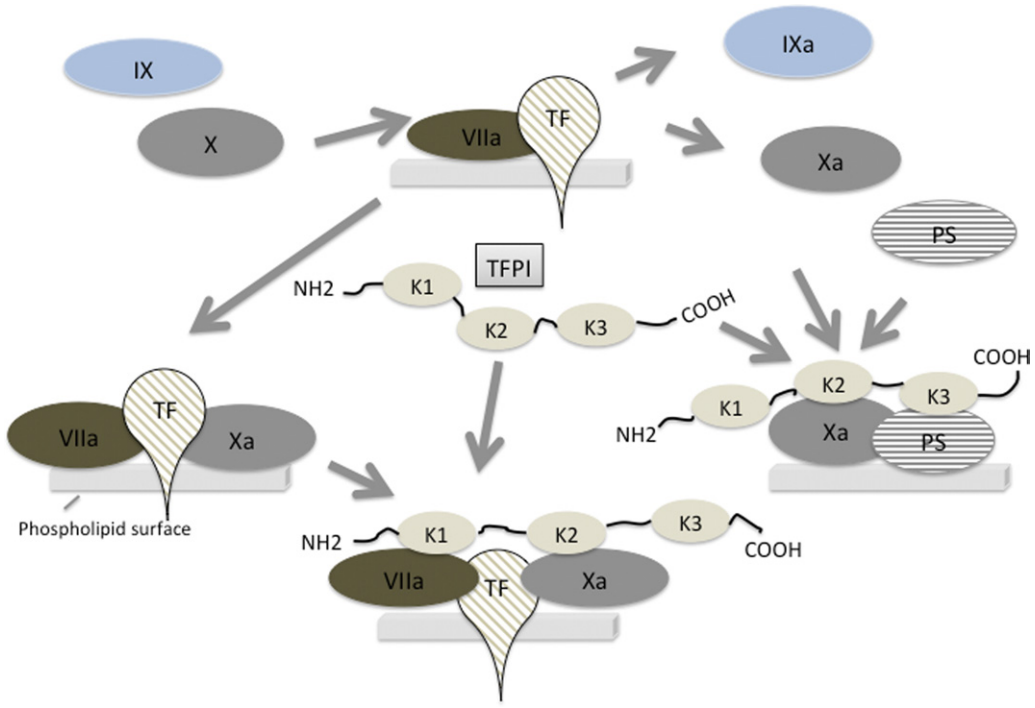

Figure 1. The functions of TFPI. The TF-VIIa complex is able to activate FIX to FIXa and stimulate the intrinsic pathway as well as activate FX to Xa (top center). TFPI binds to the tertiary complex of TF-VIIa-Xa, which is produced during the activation of Xa (center left). This binding forms the quaternary complex, with K1 binding to and inactivating VIIa and K2 binding to and inactivating Xa (bottom). TFPI is also able to bind via K2 to Xa and PS via K3, forming an inhibitory complex (center right). TFPI, Tissue factor pathway inhibitor; TF, tissue factor; K1, K2, K3, Kunitz domains 1, 2, and 3. (Adapted from Broze GJ, Jr, Girard TJ. Tissue factor pathway inhibitor: structure-function. Front Biosci 17:262-280, 2012.)

total TFPI ELISA measures binary and quaternary TFPI-Xa complexes, intact and truncated forms of TFPI as well as complexes of TFPI with TF and FVIIa. The activity assay uses thawed anticoagulated whole blood in which the cells are lysed and cellular debris removed. The supernatant is then incubated with TF-FVIIa and FX. Residual TF-VIIa activity is based on measuring FXa activity and TFPI is measured from this with a standard curve.[1] Interpretation of results is method specific, and levels cannot be compared between different types of tests.[16]

TFPI in Disease

There are no known deficiencies of TFPI, suggesting it is essential for life.[12] Increased levels of TFPI have been observed in certain cancers, sepsis, uremia, unregulated diabetes, and in patients with hyperlipidemia.[17-21] Depending on the disease process, the measurement of TFPI may be used as a biomarker and the trends of increase or decline used as a prognostic indicator.[20] In septic humans and those with disseminated intravascular coagulation (DIC), an increase in mortality is noted if the production of TF is not adequately balanced by the production of TFPI.[22]

Several types of human neoplastic cells are known to alter the expression of TFPI,[23-25] contributing to a poor prognosis. A recent study determined that in certain human breast cancer models, TFPI was downregulated.[26] Also noted was that solid tumors, versus hematological cancers, trend toward higher levels of TFPI. This has been speculated to be potentially protective against microthrombosis and organ failure in certain cancers.[20]

There are limitations when evaluating studies on the role of TFPI in disease states because there is a wide range of TFPI among normal individuals, and many of the studies on TFPI and risk of thrombosis are retrospective or case-controlled and do not address whether the low levels of TFPI are the cause or the effect. There remains an insufficient number of prospective studies evaluating the role of TFPI in the development of venous thromboembolism.[1]

Therapeutic Applications of TFPI

Given the dominant role of TF in certain disease states it would seem intuitive that TFPI could be used therapeutically as an anticoagulant as well as an anti-inflammatory agent. People with low circulating levels of TFPI have been shown to be at increased risk for venous thromboembolism.[27] A study determined that TFPI was increased after oral supplementation with a fatty acid in people with chronic atherosclerotic disease, a disease characterized by high levels of TF. This increase in TFPI could promote its use as a mild antithrombotic agent to help to dampen the TF pathway.[28] Recombinant TFPI (rTFPI) has been examined for its role as an antithrombotic agent, and it was determined that local administration (injections of rTFPI near a created wound) prevented thrombosis and was equivocal to heparin and dextran.[29] Several studies have evaluated recombinant TFPI supplementation in animal models.[30,31] Human studies focus importance on the levels of TFPI secondary to heparin therapy and use those levels as a marker for postoperative bleeding.[32,33]

The use of rTFPI showed great promise in animal models of sepsis and DIC. A study evaluating intravenous rTFPI determined that the rTFPI prevented thrombosis and progression into DIC in a rabbit model.[34] Administration of rTFPI reduced mortality in baboons and rabbits with *Escherichia coli*–induced septic shock.[35,36] Unlike animal studies using rTFPI, the use of TFPI in humans currently shows no benefit as a therapeutic agent in sepsis. Initially phase I and II studies were promising[37] and rTFPI was safe in humans, but when taken to a phase III level, there was no survival benefit to its use in sepsis.[38]

Antithrombin

Background

AT, previously termed antithrombin III, is a plasma-derived glycoprotein. AT is a serpin (serine protease inhibitor) and shares approximately 30% homology in amino acid sequence with other serine pro-