Fibrinolysis: Why Clot Breakdown May be Just as Important as Making the Clot  
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Diagnosis and treatment of coagulation disorders associated with many veterinary diseases have been well studied, but the degree to which disorders of fibrinolysis may contribute to bleeding in veterinary patients is unknown. Recent data in the human and experimental literature suggests that the fibrinolytic pathway may be a useful target in patients with bleeding disorders and thrombotic disease.

A Review of hemostasis

Coagulation

The coagulation cascade consists of sequential activation of a series of coagulation factors. Most of these are serine proteases, but some of the factors involved in coagulation are glycoproteins (factors VIII and V) or transglutaminases (factor XIII). Most are synthesized in the liver.

The traditional model of the coagulation system is presented in abbreviated form in Figure 1. It is described as 2 arms representing the intrinsic (or contact pathway) and extrinsic (or tissue factor pathway) cascades, which then enter the common cascade, concluding in the production of thrombin, which catalyzes the conversion of fibrinogen (Fg, factor I) into fibrin (Fb, factor Ia). This model represents the processes leading to measureable coagulation resulting from in vitro testing, but in recent years, a more physiologic model of the coagulation system has been proposed.

The cell-based model of coagulation more likely represents the actual processes occurring in vivo. It begins with activation of platelets (primary hemostasis), which provide a stimulus and a surface upon which the coagulation cascade can be initiated and amplified (secondary hemostasis).

Coagulation begins with primary hemostasis, consisting of activation of platelets by exposure of subendothelial collagen. In addition, von Willebrand factor (vWF) is released from the endothelium and from activated platelets, and increases adhesion of the platelets to the damaged endothelium. Activated platelets release substances stored in cytoplasmic granules that contribute to activation of more platelets. The result is a shape change in the platelets to a stellate conformation, and the platelets then become crosslinked via fibrinogen bound to surface receptors, forming an initial platelet plug. Secondary hemostasis, also referred to as the coagulation cascade, is triggered after platelet activation. The initiation phase of coagulation is accomplished via the tissue factor pathway (extrinsic cascade). Damaged endothelial cells, activated platelets, and subendothelial fibroblasts express tissue factor (TF), which activates factor VII, producing VIIa, ultimately resulting in the production of thrombin (IIa) via the common cascade. This process produces only a small amount of IIa, which serves mainly to activate factors V and VIII. The amplification phase of coagulation (Figure 3) is largely the result of activation of the contact pathway (intrinsic cascade). The trace IIa produced during initiation serves to activate more platelets, which express receptors for Va and VIIIa, ultimately leading to a “thrombin burst,” a massive increase in the amount IIa produced by the common cascade. This large production of IIa catalyzes both the conversion of Fg (I) to Fb (Ia) and activation of factor XIII, which initiates crosslinking of Fb and formation of a stable clot. Figure 2 shows a summary of the cell-based model of coagulation.

Anticoagulation

Several negative feedback mechanisms antagonize hemostasis and maintain localization of the coagulation process. Protein C is a vitamin K dependent anticoagulant synthesized in the liver that is activated by thrombin (IIa) bound to thrombomodulin on the endothelial cell surface. Activated protein C (APC) has its anticoagulant effect by deactivating Va and VIIIa in the presence of its cofactor Protein S. Antithrombin (AT) is produced by the liver and deactivates thrombin as well as IXa, Xa, Xla and XIIa. Its activity against Xa is markedly enhanced by heparan sulfates on the endothelial cell surface and by exogenous heparin. Endogenous heparan sulfate and
some exogenous heparins (larger molecular weight) also enable AT to directly deactivate IIa (thrombin). Tissue factor pathway inhibitor (TFPI) inhibits the action of TF on activation of VII as well as TF mediated activation of IX and X. Finally, prostacyclin is released by endothelial cells, activating Gs-linked receptors on platelets, increasing intracellular cAMP levels and decreasing intracellular calcium concentrations, inhibiting platelet degranulation and activation.

**The fibrinolytic system**

**Activation of fibrinolysis**

Clot breakdown (fibrinolysis) is initiated by release of plasminogen activators, predominantly tissue plasminogen activator (tPA) from endothelial cells and urokinase-like plasminogen activator (uPA), which circulates in plasma and is produced by various tissues. tPA binds to fibrin and predominantly activates plasminogen bound to fibrin in the area of a clot. Fibrinolysis is enhanced locally by protein C via a dis-inhibition mechanism in response to clot formation. Protein C is activated by the thrombin-thrombomodulin complex as described above. Activated protein C (APC) enhances fibrinolysis by inhibiting both activated TAFI and PAI-1, the major inhibitors of fibrinolysis. Kallikrein activates circulating pro-uPA into active uPA, which also leads to plasmin generation from plasminogen. This process is initiated by factor XIIa from the coagulation cascade, which catalyzes production of kallikrein from pre-kallikrein. These processes all occur locally in the area of clot formation, stimulating clot breakdown.

**Inhibition of fibrinolysis**

Several endogenous mechanisms antagonize fibrinolysis. A major antagonist of fibrinolysis is thrombin-activatable fibrinolysis inhibitor (TAFI), which is produced by the liver and circulates in the plasma in an inactive form. It is activated by the thrombin-thrombomodulin complex on the surface of endothelial cells in the area of a clot and directly inhibits the activity of plasmin. The other major fibrinolysis inhibitor is plasminogen activator inhibitor-1 (PAI-1), which is present free in the circulation and inhibits the activity of both tPA and uPA. There is evidence that PAI-1 is produced by endothelial cells, liver, and/or platelets. In addition, there is recent evidence that significant production may also occur in adipocytes. Less important inhibitors of fibrinolysis include α-2 antiplasmin and α-2 macroglobulin. They are synthesized in the liver and by platelets and directly inhibit the actions of plasmin. Factor XIIIa also catalyzes the binding of α-2 antiplasmin to fibrin, making the fibrin more resistant to breakdown by plasmin. There are also 2 main classes of pharmacologic agents that inhibit fibrinolysis. Aprotinin is a serine protease inhibitor that directly inhibits the activity of plasmin. At higher concentrations, it also inhibits kallikrein, which reduces the production of plasmin directly. The other class of fibrinolysis inhibitor drugs is the lysine analogs (epsilon-aminocaproic acid, EACA, and tranexamic acid, TEA). These drugs block lysine binding sites on plasminogen that are essential for its activation to plasmin, decreasing plasmin production.

**Species differences in fibrinolysis**

There is strong evidence that compared to humans, dogs demonstrate enhanced fibrinolysis. This was first noted when investigators attempted to develop a canine model of pulmonary thromboembolism (PTE), but found that the emboli lysed within hours both in vitro and post mortem in dogs compared to emboli that frequently last months to years in people. There is also evidence in the literature that horses may have reduced fibrinolytic activity compared to people, demonstrated by lower basal plasminogen activity and increased α-2 antiplasmin and PAI-1 activity in adult horses and foals. In addition, horses require 10-fold higher concentrations of tissue plasminogen activator (tPA) to induce fibrinolysis in an *in vitro* assay compared to humans. The fibrinolytic system in cats has been poorly studied.

**Laboratory evaluation of fibrinolysis**

Common laboratory tests of coagulation, such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT), are insensitive to the status of the fibrinolytic system, and tests to evaluate the fibrinolytic potential in an individual patient are limited. Options to investigate the fibrinolytic system clinically include individual fibrinolytic factor assays, in vitro clot lysis tests, and viscoelastic testing methods.

Viscoelastic coagulation tests, such as Thrombelastography (TEG) and Rotational Thromboelastometry (ROTEM), were designed to provide a more global view of hemostasis and fibrinolysis than traditional testing. They are in vitro tests in which whole blood or plasma are allowed to clot in a cuvette and the strength of the clot over time is measured using the torsion detected on a pin submerged in the sample. Clot formation can be accelerated by the addition of various activators, and clot lysis can be measured if the assay is allowed to run for sufficient time.

ROTEM and TEG measures of fibrinolysis include the % of clot strength remaining 30 or 60 minutes after maximum clot strength (TEG CL30, CL60) and the % reduction of the maximum clot area at 30 or 60 minutes after maximum clot strength (TEG LY30, LY60). However, because in vitro clot lysis proceeds slowly due to plasma anti-fibrinolytic agents (such as α-2 antiplasmin) and the lack of locally produced pro-fibrinolytic agents that would enhance fibrinolysis in areas of clot formation (tPA and APC-induced inhibition of TAFI and PAI-1), in vitro fibrinolysis proceeds slowly and may not be detectable before the samples dehydrate. More recent modifications of these assays using added recombinant tPA to accelerate fibrinolysis, allowing measurable lysis to occur quickly, have been validated in human patients, shown to be reliable and reproducible, and were reflective of plasma concentrations of
PAI-1. A similar assay has been applied in dogs and cats by the author, and may offer a more global, point of care test for diagnosis of fibrinolytic disorders.

**Hyperfibrinolysis**

There has been very little investigation of disorders of fibrinolysis in veterinary patients, but there are several well-described syndromes of hyperfibrinolysis in people. It is likely that similar disease processes occur in other species, but further investigation is needed. The following is a review of these disorders in humans and the small amount of veterinary data available.

Hyperfibrinolytic disorders may be congenital or acquired, and commonly lead to spontaneous bleeding or worsened hemorrhage in patients with other underlying bleeding disorders. Congenital hyperfibrinolytic disorders are exceedingly rare in people and, to the author’s knowledge, have not been reported in veterinary species. Acquired hyperfibrinolysis is more common in humans. Hepatic cirrhosis is the most common disorder leading to hyperfibrinolysis in people, and is due to increased plasma tPA from decreased hepatic clearance as well as decreased hepatic production of α-2 antiplasmin. Combined with the coagulopathy and thrombocytopenia commonly seen in this disease, severe bleeding can result. Recent data in humans showed objective evidence of hyperfibrinolysis in patients with cirrhosis. In some cases refractory to other therapies, dramatic responses to EACA have been reported, suggesting that hyperfibrinolysis may be important in this clinical bleeding disorder. Neoplasias, such as acute promyelocytic leukemia (APL) and some solid tumors, most commonly metastatic carcinomas, can demonstrate hyperfibrinolysis in conjunction with DIC. Neoplastic promyelocytes produce leukocyte elastase, which can inactivate α-2 antiplasmin, and myeloid leukemic blasts have been shown to produce tPA and uPA in vitro. Clinically, there are case reports of bleeding patients with APL responding to treatment with EACA. Snake venoms, especially eastern and western diamondback snakes, may contain a tPA-like substance or may stimulate endothelial cells to produce tPA. Marked consumption of α-2 antiplasmin and PAI-1 are measurable in these patients. However, due to the high risk of DIC in envenomated patients, the use of anti-fibrinolytic agents in these patients is not recommended.

Recently, the effect of hyperfibrinolysis on the bleeding disorder associated with trauma has been intensively investigated. Although the coagulopathy associated with trauma has been well documented, the mechanism associated with it is still poorly understood. Trauma patients with coagulopathy have a higher mortality rate (46%) than those without coagulopathy (10.9%), suggesting that this disorder is at a minimum an important marker of severity of injury and may be causally related to mortality in trauma patients. Previously, it was believed that a combination of dilution from fluid resuscitation, inactivation of the coagulation cascade from acidemia, and consumption of coagulation factors from endothelial damage were the primary causes. However, 10-34% of human trauma patients have documented coagulopathies prior to fluid administration, suggesting that dilution is unlikely the sole explanation for this phenomenon.

Recently, it has been determined that regardless of injury severity, less than 3% of patients without evidence of significant shock after trauma (patients with a base deficit ≤ 6mEq/L) developed clinically significant coagulopathy, while 19.6% of patients with base deficit > 6mEq/L had coagulopathy. These data suggest that the combination of trauma and shock increases the risk of development of coagulopathy. That same study showed that in patients with BD > 6mEq/L, the risk of coagulopathy increased as injury severity increased, documenting a combined effect of severity of injury and shock on development of coagulopathy. Although patients with worsening severity of injury generated more thrombin, worsening base deficit did not affect thrombin generation or levels of factor VII, suggesting that consumption of coagulation factors is unlikely a significant contributor to the coagulopathies documented in this study.

The degree of fibrinolysis (as determined by d-dimer and prothrombin fragment concentrations) in the trauma patients also increased with increasing injury severity in the presence of shock, but only increased in the most severely injured patients without evidence of shock. Further supporting the concept that hyperfibrinolysis is present in poorly perfused trauma patients, tPA and thrombomodulin concentrations were increased, and protein C concentrations were decreased (suggesting that more circulating protein C was converted to the active form, which is not measured by this assay) in these patients. Additional evidence supporting the concept that hyperfibrinolysis plays an important role in the poor outcomes associated with trauma patients in shock comes from the CRASH-2 trial and subsequent post-hoc and meta-analyses, which showed an approximately 10% reduction in risk of death in trauma patients treated with a fibrinolysis inhibitor (tranexamic acid). In summary, there is evidence that patients with trauma in combination with hypoperfusion commonly develop coagulopathies, which are associated with a worse outcome. These coagulopathies are most commonly due to a combination of systemic anticoagulation and hyperfibrinolysis due increased activation of protein C and release of tPA.

**Treatment of fibrinolytic disorders**

Fibrinolytic disorders in dogs and cats are poorly understood and likely under-diagnosed due to limited diagnostic options. In addition, therapeutic options for these disorders are limited due to a lack of pharmacokinetic studies and clinical trials documenting a benefit. In patients with or at risk of hyperfibrinolysis due to trauma, the lysine analog anti-fibrinolytic agents have been shown to improve outcomes in humans with no increased risk of thrombosis. Unfortunately, veterinary dosing regimens for these drugs are currently
extrapolated from human data, and recent studies suggest that these schemes may result in overdosing in horses and underdosing in dogs. However, some benefit of EACA has been reported in post-operative greyhounds, a breed that has been noted to be at risk of bleeding due to potential hyperfibrinolysis. In addition, a recent study documented hyperfibrinolysis in dogs with spontaneous hemoperitoneum. It is likely that anti-fibrinolytic drugs, which are inexpensive and safe, may prove useful in bleeding veterinary, and may represent an alternative to expensive therapies like plasma.