Prevention of Thrombosis: Does Pathophysiology of Thrombus Formation Matter?

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Pathophysiology of thrombosis

Thrombosis is defined as the pathologic formation of a blood clot inside a blood vessel. Thrombosis can occur in either arteries or veins. Importantly, the pathogenesis of the generation of an arterial or venous thrombus differs, although this distinction is not commonly considered in small animal medicine. Arterial thrombi form primarily as a consequence of platelet activation under high blood flow conditions in arteries and arterioles, and are described as "platelet-rich." Venous thrombi form under low blood flow in veins and venules and are fibrin-rich due to the activation of coagulation. Recognizing differences in what initiates a thrombus for a specific predisposing underlying disease and in an individual patient has therapeutic implications.

In this session we will review current concepts regarding coagulation and information regarding the pathophysiology of thrombus formation in the context of how it affects the choice of antithrombotic drugs.

Review of coagulation

Primary hemostasis refers to the formation of the platelet plug, while secondary hemostasis refers to activation of the coagulation cascade and the formation of a fibrin network. Clinically it is useful to separate hemostasis into these two stages because disorders of primary and secondary hemostasis have distinct clinical presentations and causes. However, during normal hemostasis, activation of coagulation and platelets occurs simultaneously. Formation of the platelet-fibrin clot is occurs during three overlapping phases, initiation, amplification and propagation. Endothelial damage initiates the formation of a platelet plug through binding of platelets to subendothelial collagen, which is facilitated by von Willebrand's factor. Tissue factor (TF) within the blood vessel wall simultaneously activates the coagulation protease cascade through the extrinsic cascade. The extrinsic cascade is comprised of TF and factor VIIa. The extrinsic cascade initiates coagulation during normal hemostasis and in many prothrombotic states. The intrinsic cascade is not currently believed to be involved in initiation of coagulation in vivo. Tissue factor is normally absent from the vascular space, being expressed by cells surrounding blood vessels such as subendothelial fibroblasts. Thus, the initiation phase of coagulation is localized to TF bearing surfaces. During this phase, coagulation is initiated by exposure of TF to plasma due to endothelial damage, or expression of TF on the surface of activated endothelial cells, monocytes and/or microparticles.

Circulating microparticles are derived from cell membranes of RBCs, platelets, megakaryocytes, endothelial cells,neutrophils and monocytes. They express cell surface molecules that are derived from their cell of origin, and are able to interact with, and induce cell signaling in other cell types, including endothelial cells. Evidence suggests that activated platelets release phosphatidylserine (PS) exposing microparticles. PS is normally present on the inside of cell membranes. Activation of platelets and microparticle formation results in PS exposure on their external surface. PS has procoagulant effects as it provides the docking site for coagulation factors during the process of coagulation. Interestingly, deficiencies in platelet exposure of PS and microvesiculation result in bleeding tendencies in people and dogs. Microparticles derived from activated monocytes and endothelial cells and possibly platelets also express TF. Thus microparticles are thought to play a role in pathologic thrombosis as well. Regardless of its source, exposure of TF to plasma factor VII/VIIa initiates coagulation and results in the production of a small amount of thrombin.

The amplification phase of coagulation occurs mainly on platelets. Thrombin activates platelets, and platelet associated factor V. Factor Va acts as a cofactor for factor Xa. Together they form the prothrombinase complex that converts prothrombin to thrombin and this results in the production of more thrombin. The intrinsic pathway consists of high molecular weight kininogen, prekallikrien, and the serine proteases factor XII, factor XI, factor IX and factor VIII. In addition to platelets and factor V, thrombin also activates factor VIII and factor XI of the intrinsic cascade.

The propagation phase is driven by thrombin activation of the intrinsic pathway downstream of factor XII, and is thought to occur primarily through thrombin induced activation and formation of the tenase complex (factors VIIIa- IXa) and factor XIa. The TF-factor VIIa complex also activates the intrinsic cascade through activation of factor IX. Formation of the tenase complex, and subsequent further activation of factor X and V, results in further generation of thrombin. Of note, factor XII is not necessary for normal hemostasis as evidenced by the fact that factor XII deficient cats, people, and mice do not exhibit bleeding tendencies. Although factor XII deficiency does not increase the risk of hemorrhage, factor XII-/- mice have reduced thrombosis in a variety of models.

Large amounts of thrombin are produced in the propagation phase. Thrombin catalyzes the conversion of fibrinogen to fibrin, and activates the transglutaminase factor XIII which then cross-links fibrin and stabilizes the clot. In addition to its role in the propagation of the coagulation cascade and formation of fibrin, thrombin is a potent activator of platelets and endothelial cells via cleavage of protease-activated receptors.

Platelets and other cells release microparticles that enhance clotting by providing a membrane surface for the assembly of the prothrombinase and tenase complexes. Thus, platelets and the clotting cascade work together in the generation of a blood clot.

The major inhibitor of the extrinsic pathway is tissue factor pathway inhibitor (TFPI). TFPI is expressed by endothelium and binds to its surface. There are also small amounts of TFPI in the circulation. Another anticoagulant expressed by activated endothelium is thrombomodulin. When thrombomodulin binds thrombin its substrate specificity changes and it becomes an anticoagulant protein by activating protein C. Activated protein C with its cofactor, protein S, cleaves and inactivates factors Va and VIIIa. Antithrombin (AT) inhibits factors Xa, IIa, VIIa, IXa, XIa and XIIa. The activity of AT is dramatically increased after binding heparan sulphate, which is expressed on the surface of endothelial cells.

Fibrinolysis occurs gradually after clot formation. Activated endothelium and monocytes produce tissue plasminogen activator (tPA), which converts plasminogen to plasmin. Plasmin is an endopeptidase that cleaves fibrin which destabilizes the clot and results in the production fibrin degradation products. Inhibitors of plasmin generation and activity include plasminogen activator inhibitors, α-2 antiplasmin, α-2 macroglobulin and other protease inhibitors.

Increased procoagulant, decreased anticoagulant and impaired fibrinolytic activity may shift the hemostatic balance towards thrombosis.

Generally speaking, anti-thrombotic drugs either prevent the formation of arterial thrombi by targeting platelets (anti-platelet drugs), or they prevent the formation of venous thrombi by targeting the coagulation cascade (anti-coagulant drugs). The most prominent example of targeting platelet activation based on underlying pathophysiology of thrombus formation is myocardial infarction in people. Myocardial infarction in people results primarily from thrombus formation due to platelet activation under states of high shear stress and rate and exposure to collagen after artherosclerotic plaque rupture. TF exposure and subsequent thrombin production is also believed to contribute to platelet activation after artherosclerotic plaque rupture. Prevention of further thrombus formation in acute MI involves anti-platelet therapy such as low dose aspirin. In contrast, development of deep vein thrombosis in people is the result of activation of coagulation as an initiating event. Therefore, prevention of pulmonary thromboembolism due to deep vein thrombosis is accomplished using anticoagulant drugs such as warfarin and heparin.

It is important to keep in mind that many diseases also result in a generalized microvascular thrombosis of both arteries and veins. In human medicine, drugs that target the coagulation cascade, like heparin, are sometimes used to target coagulation and reduce levels of thrombin and thereby indirectly decrease platelet activation. In some cases drugs that target coagulation or platelets are used in combination, although the risk of hemorrhage is greater.

In small animal medicine myocardial infarction due to rupture of an artherosclerotic plaque is rare, and usually associated with a disorder of lipid metabolism. Diseases that may be associated with increases shear stress and rate include heartworm disease and endocarditis. Indeed clinical and experimental studies show platelet activation is associated with endocarditis and heartworm infection in dogs. Aspirin has been used to prevent pulmonary artery thrombosis and thromboembolism associated with these diseases in dogs.

Other cases of thrombus formation in small animal patients appear to be driven by activation of coagulation or both coagulation and platelets. These include IMHA, neoplasia and aortic thrombosis associated with certain underlying diseases. Dogs with IMHA have increased TF expression in blood, activation of coagulation, decreased AT levels and activated platelets. In the case of IMHA it seems likely that activation of coagulation initiates thrombus formation and platelets are activated secondarily however platelets may also play a primary role. From a pathophysiologic standpoint, heparin would be a logical choice as a thromboprophylactic agent in dogs with IMHA as it targets coagulation and secondarily decreases platelet activation by decreasing thrombin production. However it is not possible to evaluate the efficacy of heparin in dogs with IMHA because of a lack of controlled studies and a high likelihood of inadequate dosing. Recent evidence suggests that heparin resistance is common in dogs with IMHA and most dogs treated with standardized doses of heparin were not adequately anticoagulated. A recent study showed that individually adjusted dose heparin based on anti-Xa activity holds promise as an effective means to anticoagulate dogs with IMHA. Controlled studies comparing IAD heparin based on anti-Xa activity compared to antiplatelet drugs such as ultralow dose aspirin and clopidogrel are needed.

In people, the pathophysiology of aortic thrombosis (AT) appears to vary with the underlying cause. Aortic thrombosis may occur secondary to mural damage such as artherosclerosis or aneurysm with associated occulsion of the aorta. However AT is also associated with disorders where there are no underlying intimal lesions. These patients have underlying coagulation disorders. Patients with atrial fibrilliation are also at risk for AT due to venous stasis and activation of coagulation in the left atrium. Aortic thrombosis also occurs in patients with vasculitis from various causes. Thus the mechanism of aortic thrombus formation differs depending on the underlying disease in people.

Aortic thrombosis has been described in dogs with protein losing nepropathy, hypothyroidism, aortic neoplasia, diabetes mellitus, hyperadrenocorticism and trauma and in dogs without any identifiable underlying disease. In cats, aortic thrombosis is a well known complication of hypertropic cardiomyopathy and is associated with left atrial enlargement and spontaneous echogenic contrast in the left atrium.

Venous stasis, endothelial damage and activation of coagulation is believed to play an important role in aortic thromboembolism in cats. Thrombus formation is thought to be initiated in the left atrium with subsequent embolization to the distal aorta. Controlled

prospective studies using heparin or other anticoagulants have not been performed. A prospective study evaluating the efficacy of clopidogrel as compared to aspirin is ongoing. (http://www.vin.com/fatcat/)

Decreased antithrombin levels have been shown in dogs with PLN and was a common finding in a study of dogs with aortic thrombosis. The authors theorized that endothelial damage, hypercoagulability and hypofibrinolysis contribute to the pathogenesis of AT in dogs. In a study of dogs with PLN, hypercoagulability was demonstrated using thromboelastography. Decreased AT and increased fibrinogen were also documented. However dogs with disease not associated with AT had similar coagulation profiles in that study. The relative roles of activation of coagulation and/or platelet activation in dogs with AT are not yet known but likely vary with the underlying disease process as is the case in people. Warfarin with or without aspirin was effective in preventing progression in a retrospective uncrontrolled study of dogs with AT. Future studies regarding the pathophysiology of AT formation based on underlying disease and controlled studies comparing treatments may help direct thromboprophylactic choice for individual patients with AT.

Reliable markers of increased risk of thrombosis have not been readily identified in small animal patients with predisposing underlying diseases. Studies elucidating the mechanisms of, and markers for thrombosis will help identify promising currently used and new thromboprophylactic agents for clinical trials in dogs and cats. This may lead to more effective and individualized thromboprophylaxis for our small animal patients at risk for thrombosis.

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