

ANTIPLATELET DRUGS: WHAT, WHEN, AND HOW Susan G. Hackner, BVSc, MRCVS, DACVIM, DACVECC

INTRODUCTION

Platelets are vital components of normal hemostasis and key participants in pathologic thrombosis by virtue of their ability to adhere to injured vessels and to accumulate at sites of injury. Platelets have long been recognized as primary participants in arterial thromboembolism (TE). More recently, their role in venous thrombosis has been elucidated. Antiplatelet drugs interfere with one or more steps in the platelet activation process. A range of antiplatelet drugs are currently used in human patients, where they have demonstrated a measurable impact on the risk of TE. Experience with these drugs in veterinary medicine is limited.

PRIMARY HEMOSTASIS

Primary hemostasis occurs when platelets adhere to subendothelial proteins, exposed either by injury or disease, and subsequently become activated. These activated platelets aggregate to form a platelet plug, release agonists which recruit more platelets to the growing thrombus, and provide a catalytic surface for thrombin generation and fibrin formation.

Approximately 10^{11} platelets are produced each day under physiologic circumstances, a level of production that can increase up to tenfold during times of increased need. Canine platelets have a lifespan of approximately 6 days. Although anucleate, platelets contain a variety of intracellular organelles, including the dense tubular system (DTS) and secretory granules. The DTS is the primary calcium sequestering organelle, and also plays a major role in arachidonic acid (AA) metabolism, whereby AA is converted to thromboxane (TX) A_2 . Preformed substances are stored in platelet granules. Alpha granules contain fibrinogen, growth factors, cytokines, and adhesive proteins (vWF). Dense granules contain adenosine diphosphate (ADP), serotonin, and calcium ions.

Platelet adhesion to exposed subendothelium involves multiple adhesive ligands and receptors. Adhesive properties have been shown to be dependent on local blood flow conditions – namely, high shear or low shear. In general, conditions of high shear are thought to be present in small- and medium-sized arteries; low shear conditions are present in large arteries and all veins. During high shear conditions, initial platelet adhesion is mediated largely by collagen and vWF, to form a fragile platelet monolayer. Under low shear conditions, platelet adhesion occurs via collagen, fibronectin, and laminin. After adherence, under all shear conditions, platelets undergo a shape change. This leads to activation, the process whereby a variety of agonists act to recruit additional platelets. This activation process is initiated by the engagement of a range of specific cell surface receptors and associated intracellular signaling pathways. Collagen and vWF are considered primary hemostatic agonists, whereas thrombin (generated by the coagulation cascade), ADP (released from dense granules) and TXA₂ (synthesized and released by activated platelets) are secondary, but vitally important, agonists. They induce platelet activation by initiating a pathway that elevates cytosolic free calcium and decreases intracellular cAMP. Each agonist, to variable degrees, stimulates a range of common platelet responses. These include: (1) the release of products from alpha and dense granules (ADP, serotonin, calcium, fibrinogen); (2) the initiation of the AA cascade, leading to TXA₂ release; (3) the synthesis and release of platelet activating factor (PAF); and (4) the surface expression of adhesive receptors (exposing a binding domain on the integrin $\alpha_{IIb}\beta_3$ receptor). These responses act to recruit additional platelets and induce their activation and adhesion to the platelet plug.

Platelet aggregation is mediated largely by the integrin $\alpha_{IIb}\beta_3$ receptor (formerly known as the GPIIb/IIIa complex) that facilitates interplatelet binding. Activated platelets provide a template, via the integrin $\alpha_{IIb}\beta_3$ receptor, for secondary hemostasis, leading ultimately to the formation of a crosslinked fibrin meshwork.

Negative modulation of platelet adhesion and activation is exerted by a variety of mechanisms. The intact endothelium has remarkable thromboresistant properties. Chemical inhibitors of platelets include prostacyclin (PGI₂), nitric oxide (NO), and platelet endothelial cell adhesion molecule-1 (PECAM-1). These are released constitutively from the endothelium and act locally to prevent platelet adhesion and aggregation. In addition, substances expressed on the endothelial surface degrade or inhibit platelet agonists. These include ADP-ase and thrombomodulin. Some drugs may interfere with these pathways, as exemplified by the dose-dependent inhibition of prostacyclin by aspirin. The apparent redundancy of mechanisms of endothelial thromboresistance is likely to limit the clinical consequences of such.

HYPERCOAGULATION AND THROMBOSIS

Thrombosis depends on three major risk factors: vascular endothelial injury, blood stasis, and alterations in the blood constituents to favor thrombosis. The term “hypercoagulable states” in dogs and cats refers to acquired disorders in patients with underlying systemic disease known to be associated with an increased risk of thrombosis. TE has been shown to be associated with the following conditions in dogs: protein-losing nephropathy, neoplasia, cardiac disease (dirofilariasis, endocarditis, and cardiomyopathy), necrotizing pancreatitis, immune-mediated hemolytic anemia (IMHA), hypercortisolism (naturally-occurring hyperadrenocorticism and corticosteroid therapy), diabetes mellitus, atherosclerosis, sepsis, trauma, and major surgical procedures. TE in cats is most commonly associated with cardiomyopathy and neoplasia. Other reported conditions include pancreatitis, IMHA, protein-losing nephropathy, protein-losing enteropathy, hypercortisolism, and sepsis.

While hypercoagulability represents a risk for TE, the actual incidence is variable and unpredictable. The likelihood of TE in patients with these conditions appears to be increased with severe manifestation of the disease, the presence of another concomitant hypercoagulable state/s, and the interplay of other factors that promote thrombosis (e.g., hypoperfusion, dehydration, immobility, venous catheterization, tissue injury and inflammatory cytokines).

Thrombosis can be classified as arterial (eg., aortic, cerebral, cardiac) or venous (pulmonary, portal, caval). Platelets are primary participants in arterial thrombosis, where the initiating event is generally intimal disruption. These thrombi are platelet-rich, and are referred to as ‘white thrombi’. Venous thrombi are referred to as ‘red thrombi’ as they are platelet-poor and contain primarily red cells and fibrin. Recent studies, utilizing radiolabelled platelets, however, have shown that early venous thrombi are indeed platelet-rich, and that platelets are extruded from the thrombus as it matures.

INDICATIONS FOR ANTIPLATELET THERAPY

There are 2 situations in which antiplatelet therapy should be considered: (1) secondary prophylaxis, in a patient that has had a TE event; and (2) primary prophylaxis, in the patient considered at risk.

The patient who has suffered a TE event is always at risk for additional TE episodes and/or propagation of the existing thrombus. Management, therefore, should be focused to prevent such occurrences, whether or not thrombolytic therapy is administered. This is achieved by the use of anticoagulants and/or antiplatelet drugs. Traditionally, antiplatelet drugs have been recommended to prevent arterial thrombosis, and anticoagulants to prevent venous thrombosis. Antiplatelet drugs are certainly indicated in patients that have had arterial TE. A multitude of studies in human patients have shown significant thrombotic risk-reduction with the use of antiplatelet therapy. The Antithrombotic Trialists meta-analysis (144,000 patients) found that antiplatelet drugs reduced the odds of stroke, MI, or vascular death in secondary prevention by >25%¹. There is also an indication for adjunctive antiplatelet therapy in patients with venous TE. Studies in human patients with venous TE demonstrate lower morbidity and mortality when antiplatelet drugs are added to the therapeutic regimen.

The mortality rate of TE is substantial, with some deaths occurring before the diagnosis can be confirmed and effective treatment implemented. Moreover, diagnosis is difficult in some cases, and treatment of established TE is not universally successful. This makes primary prophylaxis imperative in the patient at risk for TE. Objective determination of TE risk in veterinary patients, however, is exceedingly difficult. The incidence of TE in specific diseases is largely unknown, and there are few available laboratory tools to confirm hypercoagulability. Assessment of risk remains largely subjective.

Undoubtedly, the single most convincing evidence of risk is a prior TE episode. Prophylactic drugs are indicated until the cause is reversed. In many hypercoagulable patients, multiple risk factors are present, and the risks are cumulative. Such a situation should prompt consideration of prophylaxis. Severe IMHA, severe pancreatitis and sepsis are indications for prophylactic drugs. Risk stratification, however, is subjective. Nephrotic syndrome is a significant risk for TE. Determination of ATIII level can assist in risk assessment. Risk may also be estimated by the severity of protein loss, the existence of potentially contributing factors, and any history of prior TE events. Patients with hyperadrenocorticism or diabetes mellitus appear to have a low incidence of TE, and the need for prophylactic drugs in these patients is doubtful. Risk increases, however, when another thrombophilic condition is applied. In humans with hyperadrenocorticism, TE risk is correlated with systemic hypertension. This may also be true in dogs. That is, while prophylaxis is not generally indicated in the dog with uncomplicated Cushing’s disease, the patient which is to undergo surgery, which has hypertension or protein-losing nephropathy, or which has had a prior TE episode, is a candidate for prophylaxis.

The choice of drug/s for primary TE prophylaxis depends on: whether the condition is associated with arterial or venous thrombosis or both, the anticipated duration, route of administration, and the compliance and finances of the owner. Since

most hypercoagulable states in animals can result in either venous or arterial thrombosis in an unpredictable fashion, the concurrent use of anticoagulants and antiplatelet drugs has merit. In conditions associated with arterial thrombosis (atherosclerosis, cardiac disease), antiplatelet agents should be the first line of drugs. In all others, antiplatelet drugs should be considered as adjunctive agents with anticoagulants, where possible, or alone where anticoagulant therapy is not feasible. A recent retrospective study indicated improved outcomes in dogs with IMHA when low dose aspirin (with or without heparin) was included in the treatment regimen².

ANTIPLATELET DRUGS

Aspirin

Aspirin irreversibly inhibits prostaglandin-H synthase-1 (PGHS-1), suppressing the synthesis of TXA₂. Platelet activation results in the liberation of AA. PGHS-1, an enzyme with 2 catalytic sites, metabolizes AA to PGG₂ and then to PGH₂. Thromboxane synthase then modifies PGH₂ to form TXA₂. TXA₂ is released by platelets and stimulates specific surface receptors, which leads to the release of intracellular calcium, and ultimately generates more TXA₂, enhancing aggregation and platelet recruitment. Aspirin irreversibly acetylates the enzyme, preventing AA binding to the COX-1 component of PGHS-1. Platelets cannot synthesize new protein and therefore the aspirin effect is maintained for the lifespan of the platelet. COX-1 activity is regenerated as new platelets are produced. In contrast, other NSAIDs compete with AA binding to COX-1, producing a reversible inhibition. Ibuprofen has been shown to interfere with COX-1 inhibition by aspirin; binding of the drug to COX-1 prevents access of aspirin to its target.

In humans, a single aspirin dose of 160 mg completely abolishes platelet TXA₂ production (measured as its stable analogue TXB₂). The same effect can be achieved with the chronic administration of daily doses of 30-50 mg. The antithrombotic effect of aspirin is saturable, such that higher doses produce no additional benefit with respect to antiplatelet effects. Onset of effect is rapid because aspirin can acetylate platelet COX-1 in the portal circulation. Moreover, despite a half-life of 20 minutes, the antiplatelet effects persist for 24-48 hours. This may limit the consequences of less-than-ideal compliance in a real world setting.

Because aspirin acetylates COX-1 in all tissues, including endothelial cells, where the enzyme converts AA into the platelet antagonist prostacyclin (PGI₂), there has long been a concern that that antithrombotic effect of aspirin could be blunted or overcome by the theoretical prothrombotic effect associated with the parallel inhibition of prostacyclin. Unlike platelets, endothelial cells have the capacity to synthesize new COX-1. Traditional recommendations, therefore, were for low doses that could completely inhibit platelet COX-1, while sparing endothelial COX-1. Human clinical trials, however, demonstrate that aspirin is effective over a wide range of doses, indicating that the protective effect of platelet COX-1 inhibition outweighs the theoretical prothrombotic effect of decreased prostacyclin production³. This may be because endothelial cells have a second COX isoform, COX-2, that can synthesize prostacyclin in the presence of aspirin. Under physiologic conditions, COX-2 is present in only a small fraction of platelets, but the number of COX-2-expressing platelets may increase in conditions of high platelet regeneration.

In dogs, an aspirin dose of 0.5 mg q12h has been shown to inhibit TXA₂, while sparing prostacyclin⁴. Higher doses inhibit prostacyclin production. The clinical efficacy of prophylaxis at higher doses, however, has not been evaluated. In cats, a dose of 81 mg/cat q72h has been traditionally used to prevent arterial TE. This dose has been shown to decrease TXA₂ production; the effect on prostacyclin is unknown. More recently, a study of aortic TE in cats revealed no statistical difference in outcome between traditional and low-dose (5 mg/cat q72h) aspirin⁵.

Long-term therapy with aspirin is associated with a modest increase in the incidence of GI bleeding. This results from inhibition of TXA₂-mediated platelet function and impairment of PGE₂-mediated cytoprotection in the GI mucosa. Whereas the former effect is dose-independent, the latter is dose-dependent. Human trials demonstrate that low-dose aspirin is at least as effective as high-dose, but significantly less gastrototoxic. In the above-mentioned feline study, the incidence of GI side-effects with high-dose vs low-dose aspirin was 22% and 4%, respectively.

Treatment failures occur with aspirin therapy; approx. 40% of human patients on aspirin therapy develop an ischemic event. There are many possible causes for this. Aspirin is a relatively weak inhibitor of platelet function. It inhibits only one pathway of platelet activation and aggregation. Moreover, platelet aggregation is only one pathway of thrombus formation. True 'aspirin resistance' refers to failure of aspirin to inhibit TXA₂ production. Potential mechanisms include: (1) decreased bioavailability; (2) competition with other NSAIDs; (3) accelerated platelet turnover, introducing newly-formed,

nonaspirinated platelets into the circulation; and (4) TXA₂ production by the aspirin-insensitive COX-2 in newly-formed platelets. The issue of aspirin resistance and treatment failure has yet to be addressed in veterinary patients.

Thienopyridines

This group comprises ticlopidine and clopidogrel. Both require metabolism by hepatic cytochrome p450 enzymes to acquire their antiplatelet activity. Active metabolites irreversibly block ADP binding to one of its receptors on the platelet surface, the P2Y₁₂ receptor. Stimulation of P2Y₁₂ mediates a progressive and sustained aggregation, and plays an important role in the potentiation of platelet secretion induced by several agonists. Receptor blockade, therefore, prevents ADP-induced activation of the $\alpha_{IIb}\beta_3$ integrin, fibrinogen binding, and sustained aggregation. Onset of action of these drugs is not immediate; platelet inhibition occurs by 2 days, and reaches a steady state after 5-7 days. As with aspirin, half-life is short, but the defect in platelet function persists for the life of the platelet. Clopidogrel has largely superseded ticlopidine in human medicine due to superior potency, more rapid onset, and a lower incidence of limiting adverse effects (neutropenia, thrombotic thrombocytopenic purpura). Several large trials have demonstrated a marginal risk reduction of vascular events with clopidogrel compared to aspirin, and a more significant impact when aspirin was used together with a thienopyridine^{1,6}.

Veterinary experience with these drugs is limited. Ticlopidine (62 mg/kg q24h) has been shown to effectively inhibit ADP-induced platelet aggregation and decrease pulmonary lesions in experimentally heartworm-infected dogs⁷. A study in normal cats showed that, at ticlopidine doses sufficient to consistently inhibit platelet aggregability (250 mg q12h), cats became anorectic⁸. Intermediate dose ranges, however, were not evaluated. Clopidogrel, at varying doses, was evaluated in 5 normal cats⁹. Results suggested that administration at doses ranging from 18.75 to 75 mg q24h, resulted in significant antiplatelet effects.

$\alpha_{IIb}\beta_3$ antagonists

When platelets become activated, the $\alpha_{IIb}\beta_3$ receptor is converted to a functional receptor, binding adhesive proteins, facilitating platelet aggregation and providing a template for secondary hemostasis. Antagonists of $\alpha_{IIb}\beta_3$, therefore, block the final common pathway of platelet aggregation, regardless of the agonist. Three classes of $\alpha_{IIb}\beta_3$ antagonists have been developed: antibodies (eg., abciximab), synthetic peptide forms (eg., eptifibatide), and synthetic non-peptides (eg., tirofiban). At doses that result in >80% receptor occupancy, platelet aggregation is significantly reduced.

Intravenous $\alpha_{IIb}\beta_3$ antagonists have been extensively evaluated in numerous trials, involving more than 100,000 human patients¹⁰. Results indicate that, while these agents have a significant beneficial effect in very specific circumstances, such as preventing ischemic complications in patients undergoing coronary intervention, their benefits in the management of other syndromes are limited. Data on the efficacy of oral $\alpha_{IIb}\beta_3$ antagonists (eg., xemilofiban) has been discouraging. Studies have failed to demonstrate a reduction in ischemic events, despite increased bleeding. In addition, excess mortality has been documented in some groups.

Reports of $\alpha_{IIb}\beta_3$ antagonist use in small animals has been limited to experimental studies. Eptifibatide inhibits platelet aggregation in feline blood in vitro, but results in cardiovascular collapse when administered intravenously to cats. Abciximab, in combination with aspirin, was shown to reduce the extent of, but not abolish, thrombus formation in a small number of experimental cats, compared with aspirin and placebo¹¹. Oral xemilofiban, alone or in combination with aspirin, has been shown to prevent occlusive thrombus formation in a canine model of arterial thrombosis¹².

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