State of the Art Review

Diagnosis and treatment of platelet hyperactivity in relation to thrombosis in dogs and cats

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Abstract

Objective – To review the mechanisms of platelet activation and options for diagnosing and treating platelet hyperactivity in relation to thrombosis in dogs and cats.

Data Sources – Prospective, retrospective, and review articles, as well as textbook chapters in both human and veterinary medicine. Articles were primarily, but not exclusively, retrieved via Medline.

Human Data Synthesis – In people, platelets are known to play a key role in the development of arterial thrombosis in numerous disease states and antiplatelet drugs are the cornerstone in the treatment of acute events and for prevention in patients at risk. For many years, aspirin was used as the sole antiplatelet drug in people, but the introduction of adenosine diphosphate receptor antagonists and integrin $\alpha_{IIb}\beta_3$ inhibitors has significantly improved outcome in selective groups of patients.

Veterinary Data Synthesis – The understanding of platelet activation in disease states has increased dramatically over the past decade. Simultaneously, a host of new methods for evaluating platelet function have been developed, which enable primarily researchers, but also clinicians to monitor the activity of platelets. Many of these methods have been validated for research purposes, but few have found their way to the clinics. Not a single correctly randomized clinical trial has been carried out with any antiplatelet drug for any indication in dogs or cats, and consequently, treatment is empiric and largely based on expert opinion or data from experimental studies.

Conclusions – The pathogenesis of thromboembolic disease is complex and multifactorial and the role of hyperactive platelets in this etiology remains to be clarified in most of the diseases associated with thrombosis in dogs and cats. Until efficacy data from well-designed studies are available, antithrombotic therapy should consist of close monitoring, good supportive care, and judicious empirical use of antiplatelet agents.

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Introduction

The purpose of this article is to review the mechanisms of platelet activation and options for diagnosing and treating platelet hyperactivity in relation to thrombosis in dogs and cats. The review was compiled from available prospective, retrospective, and review articles, as

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Abbreviations

AA	arachidonic acid
ADP	adenosine diphosphate
ASA	aspirin
AT	antithrombin
ATE	arterial thromboembolism
CADP	collagen and ADP
cAMP	cyclic adenosine monophosphate
CEPI	collagen and epinephrine
cGMP	cyclic guanosine monophosphate
COAT	Collagen and thrombin
CT	closure time
DIC	disseminated intravascular coagulation
FIP	feline infectious peritonitis
FITC	fluorescein isothiocyanate

GPRP	Gly-Pro-Arg-Pro
IMHA	immune-mediated hemolytic anemia
IP3	inositol-1,4,5-triphophate
HAC	hyperadrenocorticism
LTA	light transmission aggregometry
MPC	mean platelet component
NSAID	non-steroidal anti-inflammatory drug
NO	nitric oxide
OMBT	oral mucosal bleeding time
PGI_2	protacyclin
PIP_2	phosphatidylinositol-4,5-bisphosphate
PKG	cGMP-dependent protein kinase
PPP	platelet poor plasma
PRP	platelet rich plasma
PLE	protein-losing enteropathy
PLN	protein-losing nephropathy
PLC	phospholipase C
RIBS	receptor-induced binding site
TEG	thromboelastography
TF	tissue factor
TXA_2	thromboxane A_2
TXB_2	thromboxane B_2
ULDA	ultra low dose aspirin
vWF	von Willebrand factor
WB	whole blood
WBA	whole blood aggregometry

well as textbook chapters in both human and veterinary medicine. Articles were primarily, but not exclusively, retrieved via Medline. Articles were reviewed and where pertinent, their bibliographies were searched for additional relevant articles.

The review is divided into 3 sections. First, an overview of the mechanisms that drive platelet activation in vivo, covering the current knowledge of platelet adhesion, and intracellular signaling pathways involved in platelet activation and aggregation, as well as pathways inhibiting activation. A thorough understanding of these pathways provides the basis for understanding the mechanisms of action of existing antiplatelet agents, but also helps to identify possible targets for novel drug development. The second section covers laboratory evaluation of platelet hyperactivity, which is rapidly evolving in veterinary medicine. As the knowledge regarding platelet hyperactivity and antiplatelet treatment grows, the requirements for platelet function tests in veterinary medicine will likely include monitoring the efficacy of antiplatelet therapy and predicting the risk of bleeding in patients receiving antiplatelet agents, especially during trauma, critical illness, and surgical procedures. The final section covers current relevant evidence-based antiplatelet therapy in both small animals and people.

Hemostasis research has exploded with new information over the past 10 years. This progress has happened simultaneously with the development of molecular and diagnostic modalities, which have enhanced our ability to study biological interactions at a far more detailed level than previously accomplished. In 1996, a novel model of hemostasis was introduced, which explains hemostasis through a cell based, complex process, where the interaction of vascular tone, blood flow, endothelial cells, platelets, coagulation factors, fibrinolytic factors, and their cofactors and inhibitors results in balanced hemostasis and formation of a clot at the injured site.[1] The model is called the "cell-based model" of hemostasis and it is a dynamic model that involves cellular regulation of hemostasis in 3 phases – initiation, amplification, and propagation (Figure 1). Research facilitated by the availability of specific reagents and cell-based model systems has confirmed the key role of tissue factor (TF) in the initiation of hemostasis and has also shown that TF-bearing cells and activated platelets act as the main cellular surfaces for assembly of the procoagulant complexes.[2]

At the same time, the close link between inflammation, the innate immune system, and the hemostatic system has undeniably been established.[3,4] In inflammatory or pathological states, monocytes, endothelium, and recently platelets have been found to express TF.[5–7] Subsequent TF binding of FVIIa and FXa initiates intracellular signal transduction pathways, which induce production of transcription factors necessary for the synthesis of adhesion proteins, proinflammatory cytokines, and growth factors.[8] This activation of coagulation in inflammation ultimately leads to production of thrombin, which is a very potent platelet activator.

A direct consequence of this new knowledge has been a significantly increased understanding of the key role platelets have in the etiology of hypercoagulable states including thromboembolic disease. Many new drugs and diagnostic tests have been studied and traditional approaches to clinical syndromes are being challenged as new information comes to light. Although many patients will benefit from this new and useful information, it also poses serious challenges for both human and veterinary medicine.

Thromboembolic complications have been described in relation to numerous diseases to date and hyperactive platelets potentially play a role in the etiology in a number of these. In dogs, the relevant diseases are neoplasia,[9–13] hyperadrenocorticism,[14–17] immune-mediated hemolytic anemia (IMHA),[12,18–20] pancreatitis,[12] disseminated intravascular coagulation,[10,13] sepsis,[10,12] cardiac disease,[11,13,21–27] diabetes mellitus,[28] and hypothyroidism.[28] In cats, the current relevant diseases are cardiac disease,[21,23–27] IMHA,[25] neoplasia,[25,29] pancreatitis,[25,27] and feline infectious peritonitis.[25]
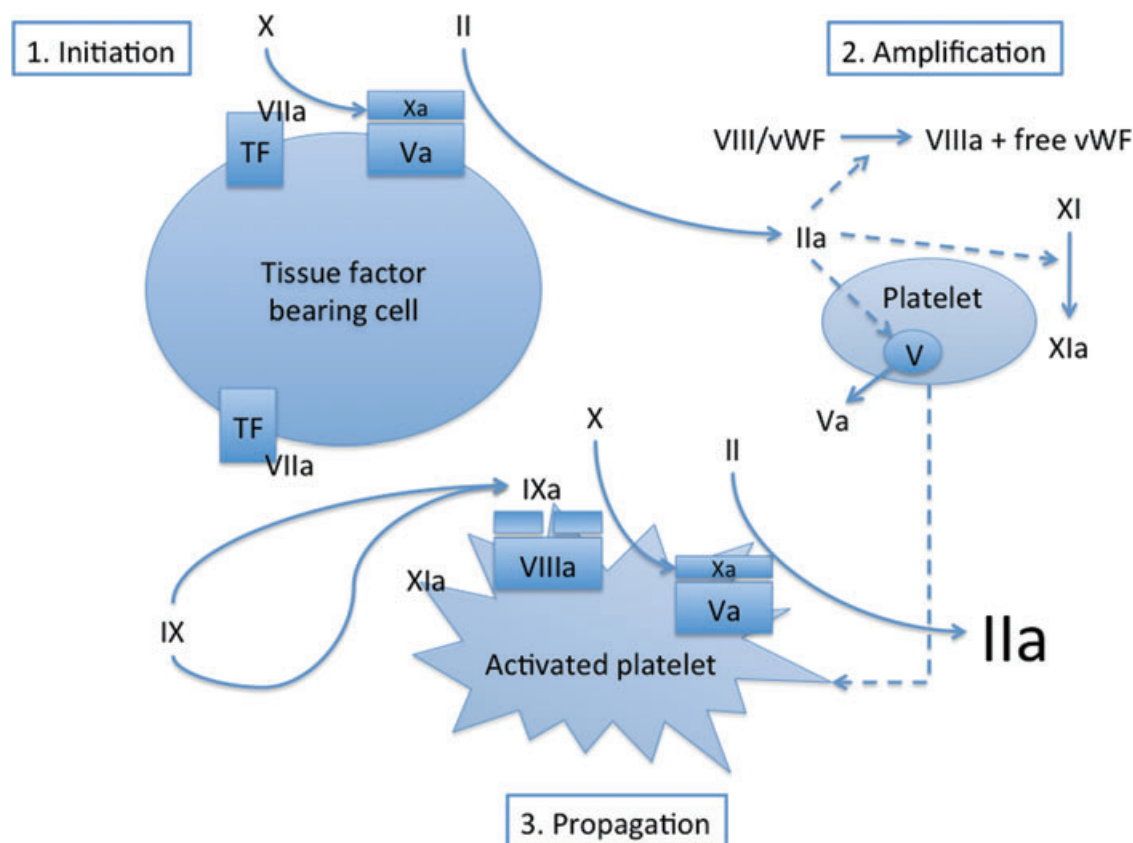
Figure 1: The cell-based model of coagulation. The 3 phases of coagulation occur on different cell surfaces: initiation on the tissue factor-bearing cell; amplification on the platelet as it becomes activated; and propagation on the activated platelet surface.

In both dogs and cats, the protein-losing diseases such as protein-losing nephropathy[30] and protein-losing enteropathy, which are often associated with thromboembolic complications, likely have a different etiology, namely low antithrombin levels.[31–33] In human medicine, there is a growing focus on concomitant risk factors, such as genetic predisposition, obesity, diet, exercise etc., which is something there has not been emphasis on in the veterinary community until now. However, it has been established that corticosteroid administration[12,22,25] and indwelling catheters[13,25] are risk factors for development of thrombosis in both dogs and cats.

Thus, it is clear that thrombosis is associated with numerous common conditions and disease states in which hyperactive platelets likely play a significant role. This awareness has led to a growing need to identify and treat both patients at risk and those, which have already suffered thromboembolic events. In order to do so, the clinician must have a comprehensive knowledge of platelet function, available diagnostic modalities, and appropriate treatment options. Therefore, this review will focus on describing the mechanisms involved in

platelet activation and signaling, laboratory diagnosis of hyperactive platelets, and finally an overview of relevant antiplatelet agents.

Platelet Activation

The mechanisms that govern the interactions between the endothelium and circulating platelets are not yet fully understood (Figure 2). Circulating platelets must tolerate turbulence and repeated contact with the normal endothelium without being activated. At the same time, they must be primed and able to rapidly identify the distinctive structures of a damaged vessel wall and instantaneously cease forward motion in the rapidly flowing blood stream. The platelets must then adhere to the site of damage despite the forces produced by the continued blood flow, and aggregate to each other, forming a stable plug of appropriate size, which can remain in place until it is no longer required, and then dissolve slowly to avoid embolization. Pathologic thrombus formation occurs when diseases or drugs subvert the mechanisms intended to prevent unwarranted platelet activation,
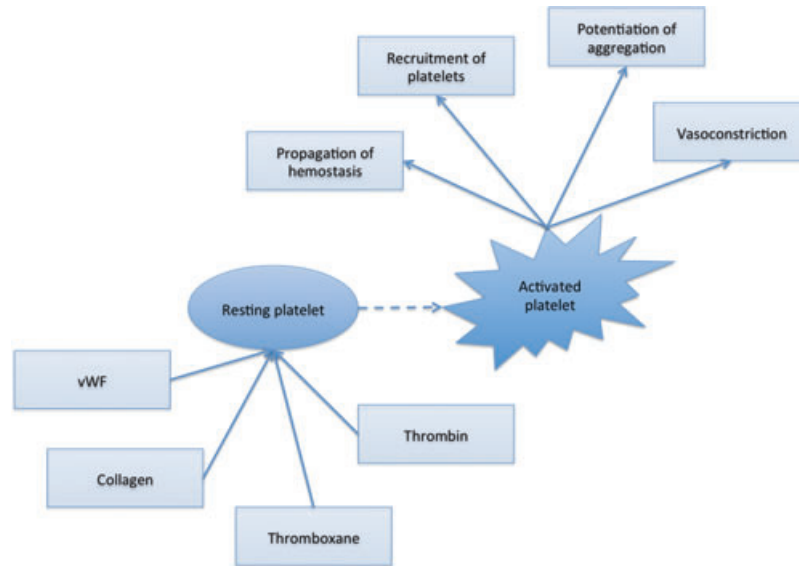
Figure 2: Key mediators of platelet activation and main effects of platelet activation. vWF, von Willebrand factor.

producing a local accumulation of platelets where none were needed.

In the setting of the hemostatic response to injury, platelets are activated by collagen or thrombin.[34] Collagen fibrils within the vessel wall become exposed to the circulating blood when the endothelial lining is damaged.[35] Under low shear conditions, in the venous system, platelets can bind directly to exposed collagen. In the arteries, the higher flow rates limit fibrin formation by rapidly diluting soluble clotting factors. Fortunately, platelets are activated more readily in the higher shear environment present in arteries, where they quickly form a physical barrier against blood loss and, at the same time, provide a surface for thrombin generation.[34,36] The higher shear rates present in arteries also help to expose the A1 domain in anchored von Willebrand factor (vWF) multimers.[37] Under high shear conditions, vWF binds to exposed collagen fibers, resulting in a shear-mediated structural change in the molecule that allows platelet glycoprotein-Ib (GP-Ib) to bind to the vWF A1 domain, thus tethering the platelet, which slows the forward progress long enough to facilitate contact between platelet receptors and their agonists.[37] Erythrocytes help this process by pushing platelets closer to the vessel wall and also in part by providing a source of adenosine diphosphate (ADP) following injury.[38,39] Pathologically, high levels of shear can activate platelets directly, but in general, various agonists present in the injured subendothelium, whose receptors are expressed on the platelet surface, activate platelets. Agonists and their respective receptors involved in platelet activation include collagen (GP VI and $\alpha_2\beta_1$), thrombin (PAR1 and PAR4), ADP (ADP; P2Y$_1$ and P2Y$_{12}$), and thromboxane A$_2$ (TxA$_2$; TP).

It is likely that all platelets are equally good at adhering and aggregating at the site of injury, but in recent years a subset of platelets, which can be observed after dual-agonist activation with collagen and thrombin (COAT) have been described.[40] These platelets are coated with a number of procoagulant proteins, such as fibrinogen, fibronectin, and thrombospondin, which give them an unparalleled ability to propagate thrombin formation. It is known that COAT platelet numbers are increased in certain disease states and it has been hypothesized that they may play an important role in the crosstalk between inflammation and thrombosis.[40]

Platelet Signaling

A simplistic overview of the most important platelet surface receptors and intracellular signaling pathways is given in Figure 3. The binding of an agonist to its receptor on the surface of the platelet initiates activation of downstream signaling pathways within the platelet, which lead to platelet degranulation, exposure of phospholipids, and exposure of the binding site for fibrinogen. The first step of the intracellular signaling pathway typically begins with the activation of the phospholipase C (PLC) isoforms contained in the platelets. Which isoform of PLC is activated depends on the agonist. Collagen activates PLCγ2 using a mechanism that depends on scaffold molecules and protein tyrosine kinases. Thrombin, ADP, and TxA$_2$ activate PLCβ using Gq as an intermediary. The PLCs subsequently hydrolyze membrane phosphatidylinositol-4,5-bisphosphate (PIP2) to inositol-1,4,5-triphophate (IP$_3$) that includes mobilization of Ca^{2+}. The rise in cytosolic Ca$_2{}^+$ that occurs during
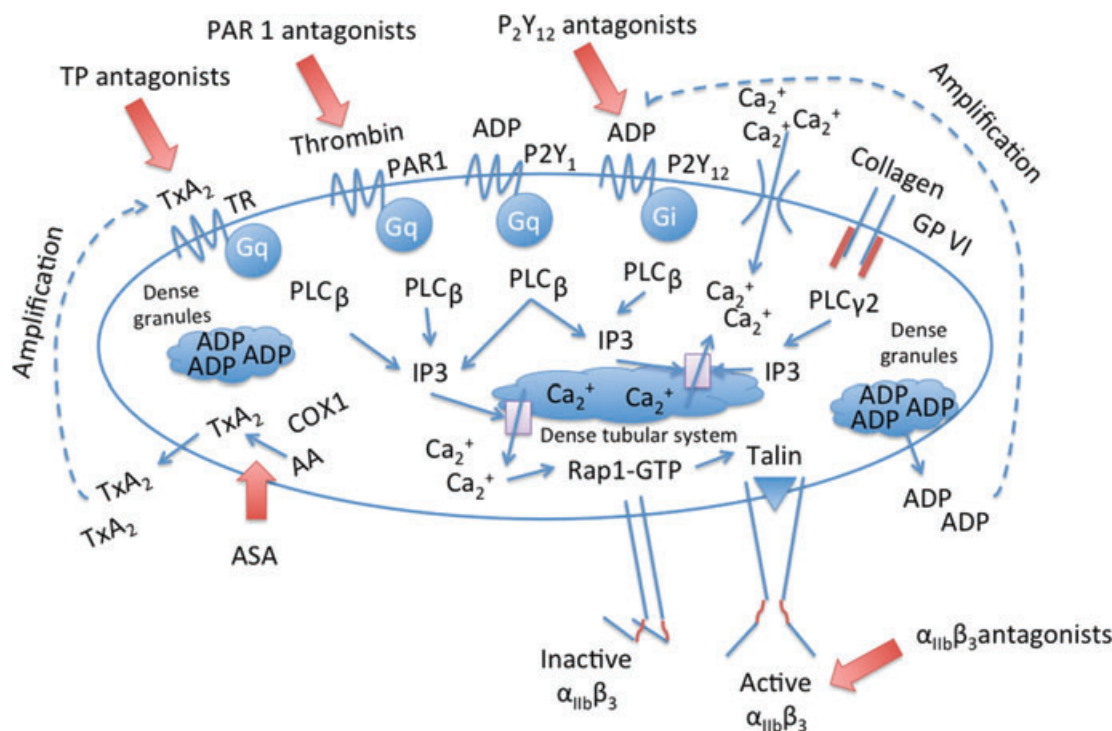
Figure 3: Major signaling pathways involved in platelet activation. $\alpha_{IIb}\beta_3$, refers to the platelet integrin that is also known as GP IIb-IIIa in older literature; AA, arachidonic acid; ADP, adenosine diphosphate; ASA, acetylsalicylic acid; COX1, cyclooxygenase type 1; Gq/i, G proteins; IP3, inositol-1,4,5-trisphosphate; PAR1, protease-activated receptor 1; PGI$_2$, prostacyclin; PLC, phospholipase C; TR, thromboxane receptor; TxA$_2$, thromboxane.

platelet activation is thought to occur as a 2-step process. First is IP^3-mediated release of Ca$_2^+$ from the platelet-dense tubular system, which triggers a secondary influx of Ca$_2^+$, driven by the steep concentration gradient that normally exists across the cell membrane. Ca$_2^+$ subsequently activates talin, via complex pathways including several signaling molecules. Talin then binds to the cytoplasmic domain of integrin $\alpha_{IIb}\beta_3$ (formerly GP IIb-IIIa), thus triggering a conformational change in the integrin, exposing a binding site for fibrinogen.[41] Other proteins that can substitute for fibrinogen include fibrin, vWF, and fibronectin. The shift of integrin $\alpha_{IIb}\beta_3$ from a low- to a high-affinity state is considered the "final common pathway" of platelet activation. In addition to forming an essential part of the hemostatic plug, platelets are delivery vehicles, secreting proteins from their α-granules and small molecules from their dense granules. These molecules have numerous established and hypothesized roles, including activation of additional platelets, promoting platelet adhesion and aggregation, reducing blood loss by initiating vasoconstriction, and, eventually, promoting wound healing.[42]

Thrombin is a strong platelet activator, which is able to activate platelets at very low concentrations. The platelet response to thrombin is largely mediated via PAR1 and PAR4 in most species, but signaling through other receptors on the platelet surface, such as GP Ib, can play an accessory role. In platelet membranes, phospholipids are not evenly distributed between the inner and outer leaflet. In resting platelets, negatively charged phospholipids, are found at the inside of the membrane and platelet activation is associated with a flip-flop move of anionic phospholipids from the inner to the outer leaflet. This exposure of phospholipids provides a catalytic surface for the procoagulant processes where the phospholipids complex with Ca$^+$ and bridge with coagulation factor complexes FIXa/FVIIIa and FXa/FVa. Binding of these coagulation factors on the platelet membrane increases their local concentration, which accelerates thrombin generation at the site of injury.[43] Platelet activation and thrombin generation, through the coagulation cascade, are thus intimately linked processes and adequate thrombin formation is not possible without activated platelets in vivo.

Endogenous Inhibition of Platelet Activation
The signal amplification and platelet recruitment mechanisms require tight control in order to limit platelet
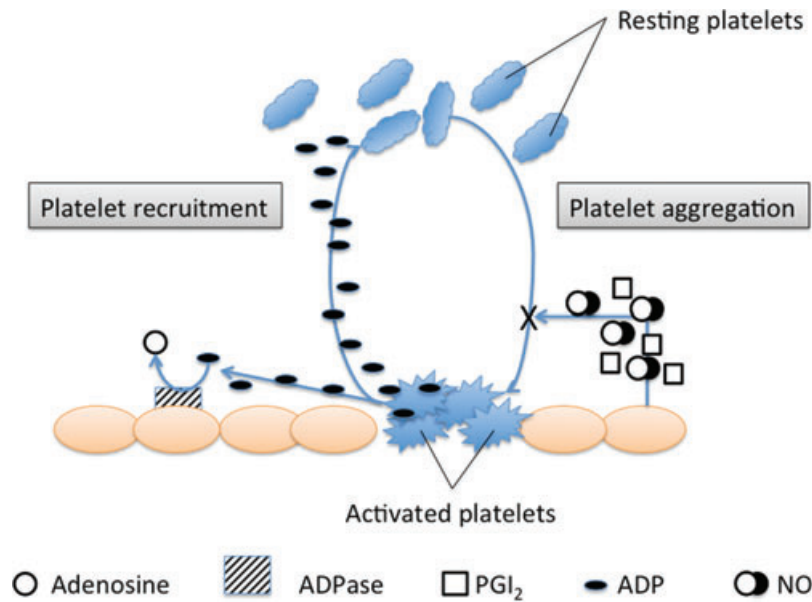
Figure 4: Endothelial cell control of platelet activity. ADPase on the surface of the endothelium metabolizes adenosine diphosphate (ADP), which is secreted by activated platelets in order to recruit additional platelets to an evolving thrombus. Endothelial cells also secrete prostacylin (PGI$_2$), which inhibits platelet reactivity by blocking cAMP. Nitric oxide (NO) from the endothelium interferes with G protein-mediated signaling, thereby inhibiting platelet activation.

aggregation to the place where it is needed (Figure 4). Vascular endothelial cells produce prostacyclin (PGI$_2$), which upon diffusion binds platelet PGI$_2$ receptors that are coupled to adenylyl cyclase. Adenylyl cyclase in turn catalyses the conversion of adenosine triphosphate into cyclic adenosine monophosphate (cAMP), which is a potent inhibitor of platelet activation.[37]

Nitric oxide (NO) is produced primarily in endothelial cells from L-arginine by NO synthase. After diffusion of endothelial produced NO through the platelet membrane, NO stimulates soluble guanylyl cyclase causing an increase in cyclic guanosine monophosphate (cGMP) and concomitant activation of cGMP-dependent protein kinase (PKG). PKG inhibits IP$_3$-stimulated Ca$_2^+$ release from the endoplasmatic reticulum, thereby decreasing the amount of cytosolic Ca$_2$.$^+$[44,45] In addition, PKG inhibits TxA$_2$ receptor function through phosphorylation, thus blocking platelet activation and aggregation.[46]

Diagnosis of hyperactive platelets

The approaches used for platelet function testing in the clinical and research settings have diverged, largely because many of the technologies now routinely applied in the research laboratory have either not been translated into technologies that can be used for clinical studies or they have not been set up in clinical laboratories. In veterinary medicine, tests of platelet function have primarily been used for the diagnosis of bleeding disorders, and to predict risks of bleeding prior to invasive procedures, but in the last few years, assays that can detect platelet hyperactivity and possibly predict thrombosis have garnered increasing interest. This section discusses currently available tests of platelet hyperfunction, their advantages and drawbacks, and their utility in veterinary clinical and research settings. Only tests on which there have been published data in the veterinary literature are included, and consequently, some of the available tests of platelet function used only in human medicine have been left out.

Platelet agonists commonly used in diagnosis of platelet function

ADP is a weak platelet agonist, and the first known low molecular weight platelet-aggregating agent. Although a weak agonist in itself, ADP plays a key role in platelet function by amplifying the platelet responses induced by other platelet agonists. There is considerable interspecies difference in platelet aggregation responses to various agonists and in canine whole blood (WB), there are some reports that ADP does not give consistent aggregation responses, possibly due to the influence of red and white blood cells on degradation of ADP in WB.[47,48]

Collagen either from bovine or equine tendon is the strongest of the typical agonists used in the clinical laboratories. Collagen-induced platelet aggregation is usually characterized by a lag phase, during which the

platelets adhere to the collagen fibrils and undergo shape change and degranulate. Canine platelets have been reported to respond in a dose-dependent manner to collagen.[48,49]

Cyclooxygenase converts arachidonic acid (AA) to TxA_2, which is a potent platelet agonist. The aggregation response induced by AA is completely inhibited by aspirin (ASA) in people; however, there are reports of variable platelet responses to AA in the veterinary literature, and this agonist may therefore not be of as great value for testing ASA and other nonsteroidal anti-inflammatory drug effects on animal platelets.[48,50,51] The use of AA as an agonist in canine light transmission aggregometry (LTA) experiments has been controversial. One study found no response to AA at 0.5 and 1 mM in any of the tested dogs, whereas others have found that canine platelets aggregate in response to AA.[48,52,53] One study found that the variable platelet responses to AA were breed dependent, and it has been suggested that the differences in sensitivity to AA in dogs may be because of inherited differences in intracellular signaling pathways.[50,54]

Thrombin is a very potent platelet agonist and in addition cleaves fibrinogen to form a fibrin clot in plasma, which makes it a challenging agonist to use for platelet aggregation testing. The synthetic peptide Gly-Pro-Arg-Pro inhibits thrombin-induced fibrin polymerization while allowing thrombin-induced platelet aggregation, and this has been used in aggregation experiments in dogs, with thrombin as agonist.[49] Platelets also respond to γ-thrombin an autolytic product of α-thrombin through the PAR 4 receptor. γ-Thrombin does not bind or hydrolyze fibrinogen and therefore, it does not activate coagulation.[55,56]

Platelet aggregation

Although its position is now being challenged by novel methods, platelet aggregation is the historical "gold standard" for testing platelet function defects. The original platelet aggregation method in platelet-rich plasma (PRP) was described in 1962 by Born who performed the experiments at room temperature.[57] Platelet aggregation is currently being performed in PRP at 37°C using a spectrophotometer, or in WB using an electrical impedance method. The aggregation pattern is classically evaluated in response to the addition of an exogenous platelet agonist such as ADP, collagen, AA, thrombin, or epinephrine.

LTA is measured in a spectrophotometer. The instrument is standardized for each subject using PRP as the most opaque setting possible (0% aggregation) and autologous platelet-poor plasma (PPP) as the most translucent setting (100% aggregation). As platelets aggregate in response to the addition of an agonist, the sample becomes clearer and an increase in light transmission through the test sample is recorded. Platelet aggregation response can be evaluated as maximal aggregation between baseline (PRP) and the theoretical 100% aggregation (PPP), or as rate of aggregation, calculated as the slope of the aggregation curve.

The classic marker of platelet hyperactivity is "spontaneous" aggregation observed in the platelet aggregometer, generally defined as occurring when PRP is stirred at 37°C without adding an agonist. A related test is to look for a leftward shift in the dose-response curves for multiple platelet agonists so that platelet aggregation consistently occurs at lower than normal agonist concentrations. Another simple way to test for spontaneous platelet aggregation in WB is by serially determining the fall in nonaggregated platelet count after stirring without adding an agonist.[58]

Although considered a very useful diagnostic and research tool for detecting platelet aggregation defects, LTA is somewhat nonphysiological because platelets are separated from the remaining blood components and aggregation takes place under low shear conditions during the test – conditions that do not accurately mimic platelet adhesion, activation, and aggregation upon vessel wall damage.

LTA can be affected by a variety of analytical and preanalytical variables. Aggregation studies are subject to poor reproducibility, which can be reduced, but not completely eliminated, with operator experience and careful attention to factors (eg, the anticoagulant used, pH, the time of day and temperature when the blood is obtained, and the time elapsed between drawing the blood and running the assays).[59–61] Careful venipuncture should be performed using a 19- to 21-G needle, preferably using a central vein (eg, jugular) with minimal stasis. Some laboratories do not consider vacutainer collection of blood as suitable for aggregation measurements due to ex vivo artifactual activation of platelets by the shear force of the vacuum. However, in other laboratories, use of vacutainer-assisted blood collection does not cause significant ex vivo platelet activation.[62] Sodium citrate (0.102 M, 0.129 M, buffered, and nonbuffered) at a ratio of 9 parts blood to 1 part anticoagulant is the classic anticoagulant for platelet aggregation testing. Low hematocrit values can affect aggregation values, as the final plasma concentration of the anticoagulant will be low compared to samples with normal hematocrit affecting the amount of free calcium available in the plasma.[63]

In the medical literature, there are a variety of opinions on whether or not it is necessary or beneficial to standardize the platelet count of the PRP used in platelet aggregations assays.[64] It has been reported that aggregation responses can vary in relation to platelet count,

and it is recommended to standardize platelet count if comparisons are made to established normal ranges of response to each agonist. However, it has been proposed that mixing PPP with PRP could influence aggregation results because PPP contains substances released from platelets and other blood cells during the centrifugation process that may influence platelet function, and a substantial higher variability has been found in aggregation responses using adjusted platelet count compared to unadjusted samples.[65]

Platelet aggregation may be carried out in a WB system using electrical impedance.[66,67] The impedance method can be used with either PRP or WB, although the WB method is the most widely used. WB aggregometry (WBA) measures an increase in impedance across 2 electrodes placed in the anticoagulated blood as activated platelets accumulate on them. Heparin, hirudin, and sodium citrate can all be used as anticoagulant. Whole-blood aggregation has many advantages, including use of smaller blood volumes, the immediate analysis of samples without manipulation, or loss of platelet subpopulations or platelet activation caused by centrifugation.

Platelet function analyzer PFA-100

The PFA-100 test is performed by pipeting 0.8 mL of citrated WB into a disposable cartridge. The blood sample is then drawn, under a constant vacuum, through a capillary tube and an aperture, which is 150 μM in diameter, over a membrane coated with collagen and epinephrine (CEPI) or collagen and ADP (CADP). Activation of platelets and adhesion to the membrane is caused by the high shear rates (4,000–5,000 s^{-1}) generated and is mediated via GPIb-vWF interaction. Subsequent release of platelet granule content and the presence of CEPI or CADP on the membrane result in platelet aggregation. When the aperture is totally occluded by aggregated platelets, blood flow through the membrane stops. This is referred to as the closure time (CT).[68] Normal reference intervals have been reported to be 53–98 seconds for CADP-CT and 92 to >300 seconds for CEPI-CT in dogs.[69] It is generally recommended that each laboratory establishs its own normal range for CT in both cartridge systems of the PFA-100.

PFA-100 CTs are sensitive to both platelet number and function. In people, there is an approximate linear relationship between CT and platelet count below 100×10^9/L. This is even more pronounced in dogs, where there is a correlation between CT and platelet count below 150×10^9/L.[70] CT also increases with decreasing hematocrit in people and dogs.[70] The exact threshold at which the CT is affected will probably vary between species; in dogs a prolongation of the CT is seen at hema-

tocrit values below 30%–40%.[70,71] Plasma concentration of vWF is an important determinant of CT in the PFA-100, since platelet aggregation at high shear rates is highly dependent on vWF. In people, it has recently been shown that plasma vWF concentration modulate PFA-100 CT to a greater extent than platelet function.[72] An association between PFA-100 CT and vWF has been shown in healthy dogs and in dogs with inherited and acquired von Willebrand disease.[70,71,73,74]

The concentration of anticoagulant is an important determinant of CT in people and in dogs. Test times are longer in 0.129 M (3.8%) citrate than in 0.109 M (3.2%) citrate, probably due to greater calcium chelation. In dogs, PFA-100 CT appears to be stable up to 8 hours after blood collection, although the authors recommend testing within 2 hours.[69] The manufacturer of the PFA-100 currently recommends a 4-hour testing window for people.

It is unclear whether shortening of the PFA-100 CT truly reflects platelet hyperfunction or increased activity, since there is a lack of data that links shortened CT to clinical evidence of thrombosis or thrombosis-related outcome. Shorter CTs have been associated with ST elevation myocardial infarcts in human patients, but data are lacking in veterinary medicine. Greyhounds have been found to have shorter CT compared to other dogs; however, this is likely linked to the higher hematocrit found in this breed.[75]

Flow cytometry

Flow cytometry using antibodies that recognize proteins on the platelet surface or the phosphorylated forms of intracellular proteins has moved from the research laboratory to the specialty clinic, but widely used research methods that allow the behavior of individual platelets to be observed under flow in vivo or in vitro have not yet made the transition.

Flow cytometry is a versatile tool for studies of platelet function, which can be used for multiple purposes in veterinary medicine. Only measurement of the activation state of circulating platelets and their reactivity will be described in this section. In this regard, flow cytometry may be used to measure the activation state of circulating platelets and their reactivity, by activation-dependent changes in the platelet surface, leukocyte-platelet aggregation, and procoagulant changes in the platelet surface and to assess antiplatelet drug effects.

Flow cytometry measures specific characteristics of cells as they flow through a flow chamber, and through the focused beam of a laser. The cells can be fluorescently labeled, typically with a fluorescently conjugated antibody, or analyzed unlabeled. When labeled cells pass through the laser, the laser light activates the fluorophore at the excitation wavelength, and the emitted fluores-

cence as well as the light-scattering properties of each cell is detected. The intensity of the emitted light is directly proportional to the amount of antibody binding to the cell.

Flow cytometric assays evaluating platelet function can utilize washed platelets, gel-filtered platelets, PRP, or WB. Use of washed platelets, gel-filtered platelets, or PRP is potentially susceptible to artifactual in vitro activation as a result of separation procedures. Use of WB requires a minimum of 2 antibodies, each conjugated with a different fluorophore: a platelet identifying antibody, to separate platelets from other cells in the blood, together with a "test" antibody recognizing the antigen to be measured. Physiologic or nonphysiologic agonists can be used to assess platelet reactivity. Samples can be stabilized by fixation before or after addition of antibodies, but fixation does interfere with highly specific antihuman antibodies. Relevant matched isotype antibodies are used as negative controls. The application of flow cytometry for evaluating platelet function in veterinary medicine is limited by the lack of commercially available species-specific monoclonal antibodies. Some platelet antigens, for example, integrin $\alpha_{IIb}\beta_3$, appear to be widely preserved across species, and most antihuman antibodies directed against this antigen are cross-reactive.[76]

There are many advantages to flow cytometric analysis of platelet function. Both state of activation of and the reactivity of circulating platelets can be assessed. Only minute volumes of blood are needed and the evaluation of function of platelets in animals with severe thrombocytopenia or anemia is possible. The activation state of circulating platelets can be judged by binding of an activation-dependent monoclonal antibody in the absence of an agonist, but because platelets that become activated are usually removed rapidly from circulation, this method tends to be unreliable and lack of detection of activated platelets does not rule out enhanced in vivo platelet activation. Inclusion of exogenous agonists allows analysis of the reactivity of platelets in vitro. Activation of platelets results in a specific functional response, such as a change in surface expression of a receptor or other antigen or bound ligand. In addition, flow cytometric detection of leukocyte-platelet aggregates and procoagulant platelet-derived microparticles, which are sensitive markers of platelet activation in humans, have been investigated as markers of platelet activation in veterinary medicine.[77] Possible markers of platelet activation include activation-dependent conformational changes in integrin $\alpha_{IIb}\beta_3$, exposure of granule membrane proteins such as P-selectin, platelet surface binding of secreted platelet or plasma proteins (ie, fibrinogen), and development of a procoagulant surface (assessed by Annexin V binding). In dogs, meth-

ods to assess platelet activation by assessing platelet surface P-selectin expression have been described.[76–78] Increased platelet surface P-selectin expression has been demonstrated in dogs with inflammatory disease and with IMHA.[79–81] It should be noted that human P-selectin crossreacts with canine L-selectin that can lead to false-positive results and so far only one monoclonal canine CD62P antibody has been produced.[82,83] Assessment of fibrinogen binding has been described in dogs using a monoclonal antibody that recognizes a receptor-induced binding site on canine fibrinogen.[76,84] Moreover, exogenous fluorescein isothiocyanate-labeled fibrinogen binding as a marker of platelet activation has been described in dogs.[76] Development of a procoagulant surface can be assessed by binding of the protein Annexin V to phosphatidylserine exposed on the platelet surface, which has been described in dogs.[78] Phosphatidylserine exposure combined with bound α-granule proteins and calcein permability identify coated platelets, a subpopulation of platelets, which are highly procoagulant. Annexin V measurement is independent of species, since it is a placental protein and not an antibody directed against a specific epitope. Leukocyte-platelet aggregates are formed via interaction between P-selectin exposed on the surface of activated platelets and the P-selectin glycoprotein ligand 1 counter-receptor on the leukocyte surface. Detection of leukocyte-platelet aggregates have been described in dogs.[76,78,81]

Mean Platelet Component

Mean platelet component (MPC) is a new platelet parameter generated by the Bayer ADVIA 120, which has been investigated as an automated measurement of platelet activity. The MPC estimates the refractive index of platelets. When activated platelets degranulate the density decreases, and the MPC then also decreases.[85] Decreased MPC has been associated with inflammatory disease in dogs, with a significant inverse correlation between platelet surface P-selectin expression and MPC.[81]

Thromboelastography

Thromboelastography (TEG) has been used in numerous veterinary studies to date.[86] As with other coagulation analyses, blood samples for TEG analysis should be collected in a standardized manner and it is recommended that samples be handled without delay after receiving in the laboratory. Additionally, studies in both humans and dogs have indicated that a set time point post sampling (eg, 30 min) should be used for serial measurements.[87,88] In veterinary medicine, standard TEG analyses are almost always performed on citrated WB samples, but citrated plasma samples can be assayed as well.[89]

In standard TEG analyses, a blood sample is loaded into a prewarmed (37°C) oscillating sampling cup containing calcium, and the cup is raised into contact with a disposable pin. The pin is connected to a torsion wire within the instrument. The cup oscillates slowly from side to side, and as a clot starts to form, fibrin strands begin to bind the cup and pin together. As clotting is amplified and propagates, more and more fibrin is formed, which causes the amplitude of the pins rotation to increase. The rotation of the pin is transmitted via the torsion wire to a computer, which continually monitors the amplitude of the pin's oscillation. As the clot develops, the computer displays a tracing, which begins as a straight line, and which upon initiation of clot formation separates into 2 divergent lines representing initiation, amplification, propagation, and fibrinolysis. The platelets contribute both to the propagation phase and to the total clot strength and the assay can be modified to isolate the platelet contribution to the overall clot strength. The modified assay is the TEG Platelet Mapping assay[a] and the results correlate well with optical platelet aggregation in people.[90] In this assay, the maximal hemostatic activity is measured by a kaolin-activated WB sample stabilized with citrate ($MA_{thrombin}$). The following measurements are performed with heparin anticoagulant to eliminate thrombin activity: Reptilase and Factor XIII (Activator F) generate a cross-linked fibrin clot to isolate the fibrin contribution to the clot strength (MA_{Fibrin}). The contribution of the ADP or TxA_2 receptors to the clot formation is provided by the addition of ADP (MA_{ADP}) or AA (MA_{AA}). In situations where patients or experimental animals are receiving platelet inhibitory medications such as ASA or an ADP receptor antagonist, the platelet inhibition in response to the agonist can then be calculated with the formula: ($[MA_{ADP/AA} - MA_{Fibrin}]/[MA_{Thrombin} - MA_{Fibrin}] \times 100$] and % inhibition = (100% − % aggregation).[91] This method is necessary to determine the amount of platelet contribution to clot formation because platelet contribution (and amount of platelet inhibition) cannot be determined by evaluating stand-alone TEG tracings, as no differences will be seen.

Recently, TEG has been used to monitor the antiplatelet effect of various nonsteroidal anti-inflammatory drugs and clopidogrel in dogs.[92,93] A recent study examined the in vitro effect of the fungal metabolite cytochalasin D and the monoclonal $\alpha_{IIb}\beta_3$ antibody abciximab on TEG analyses. The results showed that cytochalasin, but not abciximab changed TEG tracings.[94] These studies emphasize a unique feature of the TEG assay, which suggests that for selected drugs, it could be utilized in targeting therapy of the patient toward a normalization of the patients TEG tracings and

hereby tailor dosage to meet the requirement of the individual patient.

Future assays

Recent abstracts indicate that TEG including platelet mapping assays, calibrated automated thrombogram, point of care aggregation based assays (VerifyNow), and singlet platelet detection based assays (PlateletWorks) likely will become part of future tools in testing the platelet contribution to the hemostatic process.[95–97]

Antiplatelet pharmacotherapy

With the advancing knowledge of platelet biology and involvement in thromboembolic etiology in numerous diseases in dogs and cats, pharmacologic manipulation of platelet function is becoming increasingly relevant in veterinary medicine. Antiplatelet treatment has advanced significantly in human medicine over the past decade and several novel platelet inhibitors are either in clinical trials or are in development.[98] As previously mentioned, platelets behave differently in low and high shear states and therefore, the appropriate type of platelet inhibitor for the clinical situation will vary accordingly. Furthermore, it is recognized that, as with all other cells and systems, platelet function is affected by the collective impact of polymorphisms that alter protein expression and function. Such differences might, for example, account in part for reproducible variations among individuals in responses to specific agonists or drugs.[99,100] Consequently, though single-agent therapy has been the mainstay of treatment, combination therapy utilizing more than one drug class is becoming popular in human medicine and combination therapy actually appears to be the most efficacious in some of clinical settings where shear stress varies among patients.

Considering the growing awareness of the role platelets plays in the development of thromboembolic complications in various diseases, it is somewhat surprising that there is not a single report of a randomized clinical trial for any antiplatelet drugs for any indication in dogs or cats. The dog is often used as a model to study effect of antithrombotic medication, and consequently, the literature is riddled with articles on the use of antiplatelet agents in various canine thrombotic models. Those studies provide pharmacodynamic information and "proof of concept" in a highly artificial and controlled environment and at best they can be used as motivation for designing true clinical studies. The following section is therefore limited to antiplatelet agents that are in current clinical use in humans and a brief review of the current status of the use of those drugs in dogs and cats.

In general, the antiplatelet agents that are in current clinical use in humans can be divided into 3 categories. (1) Drugs that directly inhibit platelet activation by blocking the receptors for agonists such as ADP and, more recently, thrombin. Clinically, available ADP $P2Y_{12}$ receptor antagonists include clopidogrel (Plavix) and prasugrel (Effient/Efient), whereas antagonists for the PAR1 thrombin receptor are in clinical trials. (2) Drugs that inhibit platelet activation by blocking intracellular signaling or raising platelet cAMP levels. Examples include ASA and dipyridamole. (3) Drugs that prevent activated platelets from aggregating by blocking integrin $\alpha_{IIb}\beta_3$, such as abciximab (ReoPro), previously known as c7E3 Fab, tirofiban (Aggrastat), and eptifibatide (Integrilin).

Direct platelet agonist receptor inhibitors
Thienopyridines are ADP receptor ($P2Y_{12}$) antagonists. Ticlopidine, clopidogrel, and prasugrel are all prodrugs and must be activated in the liver via cytochrome P450-dependent oxidation.[101] The active metabolite irreversibly binds to the ADP receptor and causes impaired release of serotonin, thromboxane, and ADP and inhibition of ADP-mediated activation of $\alpha_{IIb}\beta_3$.[102] Due to the conversion needed, the onset of action of these drugs is dose dependent, takes a few hours, and sensitive to variations in the P450 efficiency, which can translate into drug resistance.[103,104] Newer compounds, such as ticagrelor, are direct acting and reversible $P2Y_{12}$ inhibitors and therefore, the efficacy and safety should be more predictable.

Ticlopidine is a first-generation thienopyridine, which use has largely been replaced by clopidogrel due to its unwanted side effects in people (neutropenia and thrombotic thrombocytopenic purpura). The pharmacokinetics and antiplatelet effect of ticlodipine has been studied in healthy cats, but despite a consistent antiplatelet effect at high doses, the clinical use of ticlodipine cannot be recommended in cats due to gastrointestinal side effects.[105] Ticlopidine has also been studied briefly in dogs, including an experimental heartworm study, which showed that the dogs in the treatment group had less severe pulmonary lessions at necropsy.[106,107]

Clopidogrel is a second-generation thienopyridine, whose pharmacokinetics and antiplatelet effect has been studied in both healthy cats and dogs.[93,108] In people, clopidogrel is the most used $P2Y_{12}$ antagonist, where it is recommended for prevention of further thrombotic events following acute coronary syndrome and ischemic stroke. Large studies have shown that clopidogrel provides significant protection against thrombotic episodes when administered to patients at risk of thrombosis, especially in combination with ASA.[109,110] It has recently been suggested that coadministration of clopidogrel and omeprazole may decrease antiplatelet effects and thus

clinical benefit of clopidogrel in people. The effect is believed to be due to competitive inhibition of cytochrome P450, which attenuates the enzymatic activity needed to metabolize clopidogrel to its active form.[111,112] Coadministartion of ranitidine and clopidogrel does not seem to have the same inhibitory effect.[111]

In cats, doses from 18.75 to 75 mg/cat, PO, every 24 hours resulted in significantly reduced platelet aggregation and serotonin release and a 3.9–5.35 prolongation of oral mucosal bleeding time (OMBT).[108] No significant difference between doses was identified thus indicating that a dose of 18.75 mg/cat/ 24 h or lower should be adequate in cats. A mild self-limiting diarrhea was observed in 3/9 cats receiving 18.75 mg clopidogrel/cat/24 h in another study using clopidogrel.[96] The clinical benefit of clopidogrel as an antiplatelet agent in cats has yet to be documented for any clinical indication. However, a prospective, double-blinded, multicenter study on the efficacy of clopidogrel compared to ASA in preventing arterial thromboembolism (ATE) occurrence in cats with a recent cardiogenic embolic event is ongoing.[b] The ability of clopidogrel to enhance tissue plasminogen activator-induced thrombolysis has been tested in a feline in vitro model but the authors could not document any significant effect.[113]

In dogs, TEG platelet mapping and WB aggregation have both shown a rapid inhibitory effect of clopidogrel beginning 60 minutes post treatment and with a sustained significant reduction in optical platelet aggregation at 24 hours after 1.13 ± 0.17 mg/kg.[93] Platelet function has been reported to return to normal values in most dogs within 7–8 days, though there is an individual variation in duration of effect.[93] The ability of clopidogrel to enhance tissue plasminogen activator-induced thrombolysis has been tested in a recent experimental study in dogs, comparing the effect of clopidogrel and the new reversible $P2Y_{12}$ antagonist ticagrelor in conjunction with thrombolytic therapy of myocardial infarction. The study found that ticagrelor, but not clopidogrel enhanced fibrinolysis and significantly reduced infarct size.[114] The effect of ASA versus clopidogrel in combination with the direct thrombin inhibitor melagatran has been evaluated in an experimental canine model of carotid artery thrombosis.[115] The results showed that both the use of melagatran plus ASA or melagatran plus clopidogrel provided an effective treatment regimen for prevention of primary carotid artery thrombosis, with a moderate increase in bleeding.

Studies in people have shown that combining ASA and clopidogrel treatment led to and increased risk of life-threatening or major bleeding events.[116] A small-scale prospective study in 24 dogs with IMHA compared the effect and safety of (1) clopidogrel 10 mg/kg loading dose followed by 2–3 mg/kg for 89 days alone; (2) clopi-

dogrel in combination with ultra low-dose ASA (ULDA) 0.5 mg/kg, PO, every 24 hours (ULDA); and (3) ULDA alone for 90 days. No hemorrhagic events were noted in any of the groups and the transfusion requirements were similar between groups, as were the survival rates at discharge and at 90 days.[18] Although this study showed no significant differences between groups, the groups were very small and there was no placebo group. Therefore, larger randomized clinical studies must be performed before any conclusions can be drawn on the beneficial effects of clopidogrel or ASA in dogs with IMHA.

Third-generation thienopyridines, such as prasugrel (Effient) and novel reversible $P2Y_{12}$ antagonists such as ticagrelor (Brilinta), have recently been approved both in the United States and EU for use in people. The antiplatelet efficacy, pharmacodynamics, and pharmacokinetic profile of prasugrel has already been examined in several experimental studies on dogs.[117,118] In 1 study, oral administration of prasugrel to dogs (0.03–0.3 mg/kg/d) once a day for 14 days resulted in potent, dose-related and cumulative inhibition of ADP-induced platelet aggregation. The inhibitory effects reached a plateau on Days 3–5 and thereafter were maintained during dosing. Inhibition decreased gradually after cessation of dosing with near full recovery by 7 days after last dose.[119] One study has directly compared the efficacy of ticagrelor and the active metabolite of prasugrel and clopidogrel in a canine model of thrombosis. The result showed that there a was greater separation between doses that provided antithrombotic effect and those that increased bleeding for ticagrelor compared with clopidogrel and the prasugrel analog and that the ratio of dose resulting in a significant increase in bleeding time compared to the dose resulting in 50% restoration of blood flow was also greatest for ticagrelor. The results indicate that the reversibility of $P2Y_{12}$ binding with ticagrelor may cause a greater separation between antithrombotic effects and increased bleeding, thus giving a larger therapeutic window compared to the irreversible binding of clopidogrel and prasugrel.[120]

Indirect (intracellular) platelet inhibitors

Acetylsalicylic acid (ASA) is the most widely used antiplatelet drug in both veterinary and human medicine. It is a nonselective cyclooxygenase inhibitor that exerts an antiplatelet effect through irreversible inhibition of TxA_2 formation by inhibiting COX-1 for the lifetime of the platelet. Thromboxane mediates platelet activation through the G-protein signaling pathway and studies have demonstrated that up to 70% of normal dogs have a defect in the G-protein signaling, thus questioning the utility of ASA as a general antiplatelet drug in dogs, when using empiric dosing.[54] ASA resistance is also well

documented in people and what has been shown is that ASA resistance in healthy individuals is rarely, if ever, due to a failure to inhibit TxA_2 formation.[121,122] Methods and agonists used to quantitate the antiplatelet affect of ASA have been shown to variably measure the ASA-sensitive TxA_2-dependent component of aggregation.[123] Furthermore, it should be taken into account that there is poor correlation between the results obtained with the different aggregation tests.[124] In essence, the results are highly dependent on the type of agonist and machine used, and what is often interpreted as "resistance" may in fact reflect the method chosen. Regardless of mode of action and resistance, it is irrefutable that a daily intake of low-dose ASA in people is effective in preventing secondary thrombotic events with an impressive overall 25% risk reduction in patients with disease of the coronary, cerebrovascular, or peripheral arteries.[125]

ASA is the most common antiplatelet drug used in cats for the prevention of ATE. Although ASA has been used in cats for 30 years, prospective blinded, clinical, placebo-controlled studies, on the use of ASA in cats are lacking. Multiple retrospective studies have evaluated the effect of ASA and report recurrence rates from 17% to 70%.[23,126] A relatively high-dose ASA of 75–81 mg/cat/72 h has been used routinely in cats. However, a retrospective study in 2003 of 127 cats with ATE compared safety and reported recurrence rates between high- and low-dose ASA (5 mg/cat/72 h) and found no difference in recurrence between groups and much less gastrointestinal side effects with the low dose, thus indicating the potential for low-dose ASA in cats as a safe alternative.[126] A recently published study has evaluated the inhibitory effect of ASA and meloxicam on WBA in healthy cats.[127] The cats were administered 5 mg/kg ASA every 48 hours and the effect evaluated with WBA, OMBT, postaggregation serotonin plasma concentration, and thromboxane (TXB_2) concentration in serum. Meloxicam did not have any measurable effect on any of the analyzed parameters and although TXB_2 concentration were significantly lower in the cats treated with ASA at all time points, there was no statistical difference in either WBA or OMBT. One reason for the lack of effect on aggregation could be that WBA was performed with ADP and collagen and not AA, which specifically measures aggregation response via the thromboxane pathway. However, at present, it is unknown what agonist and concentration of agonist best reflects the aggregation response in vivo and therefore more studies are needed to elucidate whether ASA is an effective antiplatelet agent in cats.

Prostaglandin depletion seems to be the main mechanism involved in the development gastrointestinal side effects of ASA, but the direct contact of ASA with the gastroduodenal mucosa can also induce damage by disrupting the gastric epithelial cell barrier.[128] Prostaglandin re-

covers much more rapidly at low doses, since deacetylation of ASA takes place at first passage through the liver and therefore the systemic circulation is no longer exposed to the ASA, whereas the platelets passing through the portal system continue to be exposed.

In dogs, ASA is often used impirically as thromboprophylaxis in diseases such as IMHA, glomerulopathies, pulmonary thromboembolism, and other conditions where thromboembolism may be anticipated. The antithrombotic dose in dogs recommended by most textbooks is 0.5 mg/kg PO, once or twice daily; however, the optimum antithrombotic dose is unknown. The antithrombotic benefit of ASA alone in dogs has not been evaluated in prospective, clinical studies. In one retrospective study of dogs with IMHA, improved outcome was observed in the group of dogs receiving ULDA as adjunctive therapy to immunosuppression with prednisolone/azathioprine with or without concomitant heparin treatment.[129] The inhibitory effect of ASA on platelet function in dogs has been evaluated ex vivo in multiple studies with equivocal results, probably due to differences in methodology, natural breed variability, and resistance.[47,53,92,130,131] Significantly reduced plasma thromboxane concentration in response to 5 mg/kg ASA treatment in dogs was documented in 1 study, whereas the reduction in thromboxane concentration did not reach statistical significance in another study with 10 mg/kg ASA treatment.[92,131] The majority of the more recent studies have documented decreased WB and PRP aggregation in response to treatment with ASA in doses ranging from 0.5 (ULDA) to 10 mg/kg; however, the study looking at ULDA could not detect any reduction in P-selectin platelet expression.[47,92,130] Prolongation of PFA 100 CT in response to ASA has been documented in some but not all studies and the results of recent studies suggest that platelet CT in dogs may be agonist dependent, as is the case in people.[47,70,131,132] Gastric mucosal lesions secondary to ASA treatment in dogs have been documented in several studies with ASA doses ranging from 10 mg/kg BID to 25 mg/kg every 8 hours.[133,134] However, in a recent study evaluating gastric side effects of ULDA in combination with prednisolone treatment, no significantly increased gastroduodenal lesions were documented as compared to prednisolone alone or placebo, but a mild self-limiting diarrhea was noted in the group receiving combination therapy.[135]

Integrin $\alpha_{IIb}\beta_3$ inhibitors

Since $\alpha_{IIb}\beta_3$ is only in its high-affinity state on activated platelets and binding of $\alpha_{IIb}\beta_3$ with fibrinogen is the final major step in platelet aggregation, $\alpha_{IIb}\beta_3$ antagonists have a strong and specific effect. The commercially available $\alpha_{IIb}\beta_3$ antagonists are abciximab, tirofiban, and eptifibatide and they are all for intravenous use only. Oral formulations were originally developed but are not used in humans due to increased mortality.[136] Integrin $\alpha_{IIb}\beta_3$ inhibitors have only been used in experimental studies in dogs and cats to date, and consequently, there is no information on their efficacy or safety in any clinical setting.

In people, $\alpha_{IIb}\beta_3$ antagonists form an important component of the pharmacological management of patients undergoing percutaneous coronary intervention, such as patients with coronary artery disease.[137,138] Bleeding complications and thrombocytopenia are relatively common side effects of $\alpha_{IIb}\beta_3$ antagonists. Drug-induced thrombocytopenia often appears suddenly and can cause major bleeding and death. Severe thrombocytopenia is seen in 0.1%–2% of human patients treated with $\alpha_{IIb}\beta_3$ antagonists within several hours after first exposure and up to 12% of patients show thrombocytopenia following a second exposure.[139]

Abciximab is a chimeric Fab-fragment of the murine antihuman $\alpha_{IIb}\beta_3$ monoclonal antibody that blocks ligand binding to $\alpha_{IIb}\beta_3$ through steric hindrance. One practical difference between abciximab and the 2 small molecule agents, tirofiban and eptifibatide, is that abciximab has a longer duration of action.[140] Abciximab has been tested in 1 experimental study in a feline thrombosis model. The study was designed as a blinded evaluation of ASA and placebo versus ASA and abciximab. The results showed that ASA and abciximab in combination were significantly more effective at preventing thrombus formation than ASA alone.[141] Abciximab has also been studied in dogs in several thrombosis models; however, these studies provide little relevant clinical information other than that the drug seems to be an effective antithrombotic agent in dogs at dosages of about 0.8 mg/kg. No real safety data exist regarding risk of bleeding or long-term complication rate at this dose.[142,143]

Tirofiban is a specific nonpeptide antagonist of $\alpha_{IIb}\beta_3$. It has been tested in a single study in a cat model of cardiac ischemia, where it was used at a loading dose of 100 µg/kg followed by 5 µg/kg/min.[144] Tirofiban has also been tested in canine thrombosis models in doses ranging from 0.3 to 30 µg/kg/min. The results showed that tirofiban had a dose-dependent antithrombotic effect but also a dose-dependent increase in bleeding time from 3.5 minutes in controls to 13 minutes at the lowest tested drug concentration and >30 minutes at the highest concentration.[145] Interestingly, tirofiban has also been used to target ultrasound contrast to the site of thrombus formation in dogs.[146]

Eptifibatide is a cyclic heptapeptide derived from a protein found in the venom of the southeastern pygmy rattlesnake. Eptifibitide can cause fatal cardiotoxicity in

cats and therefore should be used with caution.[147] In dogs, eptifibitide has successfully been used in a few experimental studies.[148]

Conclusion

The pathogenesis of thromboembolic disease is complex and multifactorial and the role of hyperactive platelets in this etiology remains to be clarified in most of the diseases associated with thrombosis in dogs and cats. The understanding of platelet activation in disease states has increased dramatically over the past decade and it is now clear that the platelets are much more than just components of the hemostatic system that are activated when there is a breach of the vascular endothelium. A host of new methods have been developed in recent years, which enable primarily researchers, but also clinicians to monitor the activity of platelets. Many of these methods have been validated for research purposes in veterinary medicine, but few have found their way to the clinics. Not a single correctly randomized clinical trial has been carried out with any antiplatelet drug for any indication in dogs or cats, and consequently, treatment is empiric and largely based on expert opinion or safety data from experimental studies on canine or feline thrombotic models. Until efficacy data from well-designed studies are available, antithrombotic therapy should consist of close monitoring, good supportive care, and judicious empirical use of antiplatelet agents.

Footnotes

a   TEG Platelet Mapping assay, Haemoscope Corporation, Niles, IL.
b   FATCAT study, http://www.vin.com/FATCAT.

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