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Antithrombotic Therapy

Stephanie Ann Smith, DVM, MS, DACVIM

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Department of Biochemistry, University of Illinois, Urbana, IL, USA

Address reprint requests to: Stephanie Ann Smith, DVM, MS, Dipl ACVIM, Department of Biochemistry, University of Illinois, 318C Roger Adams Laboratory, MC-712, 600 S. Mathews Ave., Urbana, IL 61801.

E-mail: sasmith6@illinois.edu.

A B S T R A C T

Thrombosis or thromboembolism are significant concerns in companion animals and can be associated with cardiac, metabolic, neoplastic disease processes or can be one manifestation of inflammatory, infectious, and neoplastic disease conditions. Options for thromboprophylaxis available for clinical use in small animal patients are very limited, with heparin (primarily unfractionated, but more recently low-molecular-weight forms) and aspirin predominating. Controlled studies evaluating the use of these drugs are few, but there is some limited evidence for efficacy in prevention of formation of thrombi. Use of the vitamin K antagonist warfarin has been described, but the narrow therapeutic window has resulted in a high rate of serious adverse events. In human patients, the efficacy of aspirin, heparins, and vitamin K antagonists is well documented in a variety of thrombotic conditions, but there are significant limitations to each of these options. These limitations have prompted the search for new alternatives, some of which are now in wide clinical use in humans. Although the use of some of the drugs discussed here has not yet been described in veterinary patients at risk for thrombosis, many of these agents have been evaluated experimentally in dogs, cats, or both. These new thromboprophylactic agents may soon be beneficial in management of small animal patients at risk for thrombosis.

Small animal patients may develop macrovascular thrombosis or thromboembolism associated with cardiac, metabolic, or neoplastic disease processes. Additionally, microvascular thrombosis may develop because of the coagulation activation that occurs as a result of inflammatory, infectious, and neoplastic disease conditions. For more information regarding causes of thrombotic disorders in companion animals see the article "Diseases Associated with Thrombosis" in this issue.[1]

For many decades, the primary agents for thromboprophylaxis available for clinical use in small animal patients have been heparin (primarily unfractionated, but more recently low-molecular-weight forms) and aspirin. Controlled studies evaluating the use of these drugs are few, but there is some limited evidence for efficacy in prevention of formation of thrombi. Use of the vitamin K antagonist warfarin has been described, but the narrow therapeutic window has resulted in a high rate of serious adverse events. In human patients, the efficacy of aspirin, heparins, and vitamin K antagonists is well documented in a variety of thrombotic conditions, but aspirin therapy may be associated with significant gastrointestinal side effects, and therapy with heparins or warfarin generally necessitates careful monitoring to achieve efficacy and minimize hemorrhagic complications. These limitations have prompted the search for new alternatives, some of which are now in wide clinical use in humans. Although use of some of the drugs discussed here has not yet been described in veterinary patients at risk for thrombosis, many of these agents have been evaluated experimentally in dogs, cats, or both. These new thromboprophylactic agents may soon be beneficial in management of small animal patients at risk for thrombosis.

Anticoagulants

Anticoagulant therapy may be useful in patients at risk for development or recurrence of either venous or arterial thrombosis.

Heparins

Heparin has been used clinically for prevention of thrombosis and thromboembolism for over 70 years. It has many advantages over other medications but also many limitations. A variety of heparin protocols have been extensively studied in human venous and arterial thrombotic disorders, so heparin is of proven efficacy with reasonable safety in a long list of specific conditions. Appropriate dose protocols and monitoring approaches for human patients are generally well described for various specific disorders.[2] In contrast, evidence-based medicine for veterinary patients is extremely limited regarding indications for use of heparins, which heparins are indicated, and which dosing protocols are appropriate. Unfortunately, we know very little about optimal use of heparin in veterinary species for best safety and efficacy.

Mechanism of Action. Heparins act through enhancement of the inhibitory activity of antithrombin (AT). Heparins also cause release of tissue factor pathway inhibitor (TFPI) from the endothelial cell surface into the flowing blood.[3] The exact contribution of the latter effect to the anticoagulant function of pharmacologic heparins is not entirely clear.[4]

AT acts as an anticoagulant by inactivating serine proteases such as factor Xa (FXa), thrombin, and others. The inhibition of serine proteases is very slow in the absence of heparins. When AT is bound to heparin, it changes conformation in such a way that it becomes vastly more efficient at inhibiting serine proteases. Under physiological circumstances, the endothelial cells produce heparan-sulfated proteoglycans (HSPGs), a small amount of which is expressed on the luminal surface in contact with the flowing blood. The HSPGs bind AT, which then is fully capable of inactivating the thrombin that was produced in the vicinity of the HSPG. Thrombin is resistant to AT-heparin when the thrombin is bound to fibrin. Consequently, once fibrin is formed it can act as a protective reservoir for active thrombin.[5]

Molecular Structure. Heparins are negatively charged polymer molecules containing saccharide (sugar) residues. They vary widely in their size, structure, and charge, all of which impact their potential ability to enhance the activity of AT against its target enzymes.[2,6] In order to bind to AT (and therefore exert the structural change necessary for AT function), the molecule must contain an essential series of 5 particular residues. Only a portion of molecules in most prepara-

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tions of pharmaceutical heparin contain these residues.[2,6] Short molecules containing this required sequence are capable of binding to FXa, but are not adequately long to reach the heparin-binding site on thrombin. As a consequence, short heparin molecules are better at catalyzing AT to inhibit FXa than they are at catalyzing AT to inhibit thrombin. This is why low-molecular-weight heparins (LMWH) have much greater activity against FXa than they do against thrombin.[2]

LMWHs are preparations made from sorting more heterogeneous heparin preparations (containing large, medium, and small molecules) through depolymerization methods that eliminate the largest molecules and enrich the preparation for smaller molecules.[1] Different manufacturers use different methods, so different brands of LMWH have differing ability to preferentially inhibit FXa over thrombin.[7,8] The pentasaccharide fondaparinux is designed as a synthetic molecule that is extremely specific for anti-Xa activity.[9]

Unfractionated Heparin. Unfractionated heparin (UFH) is a heterogeneous (in both size and charge) mixture of heparin molecules derived from porcine intestinal mucosa. Depending on the preparation, the mean molecular weight is around 15,000 Da, with a range of 3000 to as much as 30,000.[2] It can be administered either subcutaneously (SC) or intravenously (IV). Intramuscular injection is not recommended because of local hematoma formation. It has immediate onset of activity when administered intravenously and is rapidly absorbed when administered subcutaneously, resulting in rapid anticoagulation. Heparin molecules bind to a wide variety of proteins. As a result, the bioavailability of subcutaneous heparin can be quite variable, particularly in critical illness, in which acute phase and inflammatory protein levels can fluctuate.[10] Consequently, UFH exhibits a variable dose-response relationship necessitating close monitoring by laboratory testing.[2]

Of all the types of heparins, use of UFH is the best characterized in both human and veterinary medicine. It is both widely available and extremely inexpensive, making it an attractive option. Unfortunately, UFH has the highest variability in dose–response relationship, and consequently the greatest potential for over-anticoagulation.[2] UFH also has the highest frequency of nonhemorrhagic adverse effects.[2] The pharmacokinetics and/or dynamics of UFH have been studied in normal healthy cats[11,12] and dogs[13-15] at various doses. Dogs with immune-mediated hemolytic anemia may require much higher (and less predictable) doses of UFH to achieve a therapeutic plasma concentration.[16,17]

Low-molecular-weight Heparins. LMWHs, like UFH, are chemically and functionally heterogeneous in nature. All LMWHs are not created equal. They have distinct pharmacokinetic and pharmacodynamic profiles. They vary in potency, bioavailability, and tendency to accumulate. Consequently, one drug is not interchangeable for another.[2,18,19] As a group, LMWHs all enhance AT inhibition of FXa more so than AT inhibition of thrombin.[2] The relative effects against each serine protease are described by the ratio of anti-Xa activity/antithrombin activity. LMWHs, like UFH, induce the release of TFPI from endothelial surfaces, but to a lesser (and variable) degree than does UFH.[20] In general, LMWHs bind markedly less to proteins than does UFH. As a result, bioavailability and pharmacokinetics are more predictable. LMWHs are generally only administered subcutaneously. They tend to have longer half-lives so that less frequent dosing is required, making them more convenient. Protamine sulfate only partially reverses the anticoagulant effects of LMWHs. LMWHs tend to have a lower incidence of adverse effects than does UFH. Because these drugs are under patent and expensive to develop, they are extremely costly. Generic versions are becoming more available, but there are significant concerns regarding equivalency of LMWH preparations made through different methods.[8,18] The potential utility of generic LMWHs is an area of active debate in human medicine.

Enoxaparin (Lovenox) is a LMWH preparation from Sanofi Aventis (Bridgewater, NJ). It has a median molecular weight of 4800 Da and an anti-Xa/antithrombin ratio of 3.3. Pharmacokinetics and pharmacodynamics have been evaluated in normal healthy cats[11,21] and dogs.[22]

Dalteparin (Fragmin) is a LMWH preparation from Pfizer (New York, NY). It has a median molecular weight of 5000 Da and an anti-Xa/anti-thrombin ratio of 2.0. Pharmacokinetics and pharmacodynamics have been evaluated in normal healthy cats[11,23] and dogs.[24]

Other available LMWHs not generally used in veterinary medicine include bemiparin, certoparin, nadroparin, parnaparin, reviparin, and tinzaparin.

Synthetic Heparin Derivatives. The development of synthetic derivatives arose out of the identification of the essential pentasaccharide sequence necessary for binding to AT. These drugs bind tightly to AT, resulting in slower elimination and a consequently long biological half-life. They have predictable pharmacokinetics and dose–response relationships. These agents are incapable of catalyzing the inhibition of thrombin by AT. They also do not bind many of the other heparin-binding proteins, resulting in elimination of many of the potential adverse effects of heparins.[2] They are devoid of the ability to cause release of TFPI so there may be a difference in vivo mechanism of action as compared with heparins.[9] Unfortunately, synthetic pentasaccharides tend to be very expensive.

Fondaparinux (Arixtra) is fairly new to the human market from Sanofi. Direct comparisons have indicated similar or better efficacy profiles to various LMWHs, but no reduction in the risk of bleeding.[9] It has been administered to dogs in experimental models.[25]

Adverse Effects of Heparin Use. The most common adverse effect of heparin therapy is bleeding. In humans, LMWHs are associated with less bleeding because of more predictable pharmacokinetics.[2] Other potential adverse effects are a consequence of the ability of heparins to bind noncoagulation proteins. These include heparin-induced thrombocytopenia (HIT) and osteoporosis. These adverse effects are less common with LMWHs and synthetic pentasaccharides than with UFH.[2]

The most feared adverse effect in humans is HIT. The more severe form of HIT is an immune-mediated event that occurs because of antibodies against a complex between heparin and platelet factor 4. It is associated with profound thrombocytopenia and thrombosis with severe morbidity and mortality.[2] This form has not been specifically reported in dogs or cats, but thrombocytopenia (of a variety of possible etiologies) is common in patients receiving heparin. Osteoporosis is generally a problem with very long-term heparin therapy (particularly in pregnant individuals).[2] It has not been reported in the literature for veterinary patients, but the author observed pathologic fracture with loss of bone density in a cat receiving heparin for 18 months.

Monitoring Heparin Therapy. The traditional monitoring method is to evaluate the activated partial thromboplastin time (aPTT) for adequate prolongation. Because of variations in the composition of aPTT reagents made by different manufacturers,[2] there can be significant variability between the prolongation of aPTT as measured by different reagents, making it difficult to delineate specific target prolongation ranges as "therapeutic."[26] Furthermore, the relationship between heparin concentration and prolongation of aPTT is not always linear. Additionally, because heparins cause release of TFPI from endothelial cells, measurement of in vitro heparin concentration may not adequately reflect degree of in vivo anticoagulation. Despite all of these unknowns, veterinary clinicians tend to use UFH with a target prolongation of 1.5- to 2.5-fold.[27] Although this target was extrapolated from human medicine[12,28] it has not been validated for dogs or cats.[11,27] Note that LMWHs and heparin derivatives do not reliably prolong the aPTT.

Heparin activity in plasma samples containing any type of heparin or heparin derivative can be measured by assessing the anti-FXa activity in a chromogenic assay.[14,29,30] This assay is available commer-

cially through Cornell University (http://ahdc.vet.cornell.edu/sects/Coag/) and in many local human hospital laboratories (with additional sample dilution required).[16] This test has reasonable performance characteristics, and a direct linear dose–response relationship between heparin concentration and inhibitory activity.[29] Although the chromogenic assay is highly specific for heparin activity against FXa, it does not necessarily reflect in vivo biological activity for several reasons. Variation in patient AT status can impact the biological effect of a given dose of heparin. Additionally, because the test is specific for anti-FXa activity, it does not reflect any impact of in vivo activity against thrombin (especially for UFH) or any impact of TFPI release. However, because therapeutic plasma concentrations have been well defined based on studies of outcome in large numbers of patients,[2] this test is very helpful in targeting therapeutic plasma concentration in humans. The relationship between plasma concentration (as measured by anti-FXa activity) and outcome is less well defined for veterinary species.

Thromboelastography is a real-time dynamic method that evaluates the time course of clot formation in whole blood. Several studies have described the impact on thromboelastography tracings for heparinized animal blood.[11,31] Note, however, that results are artifactually impacted by anemia,[32] which is common in veterinary patients receiving heparin.

Specific Data on Heparin for Use in Veterinary Medicine. Several studies have evaluated the pharmacokinetics of various heparins in dogs and cats. Note, however, that because all heparins and heparin derivatives are indirect inhibitors of coagulation (via their activity on AT), some variability in dose response is expected as a consequence of the availability of AT. Because of the ability of heparins to bind many off-target proteins (often increased in disease states),[10] the dose–response relationships are likely to be much less predictable in sick animals than they would be in healthy individuals. No published studies have directly evaluated pharmacokinetics in sick populations, although studies have reported wide inter-individual variation, with higher doses required for UFH in dogs with immune-mediated hemolytic anemia (IMHA)[16,17] and cats with arterial thromboembolism (ATE).[33]

Pharmacokinetics and Pharmacodynamics for Normal Dogs. UFH at 200 U/kg as a single SC injection indicated adequate anticoagulation with a duration of up to 6 hours.[12,14] When administered as repeated SC injections of 500 U/kg either every 8 hours or every 12 hours, results suggested that UFH at an initial dose of 500 U/kg followed by reduced doses every 12 hours was effective.[13] Repeated SC injections of dalteparin at 150 U/kg every 8 hours indicated adequate heparin levels.[24,30,34]

Pharmacokinetics and Pharmacodynamics for Normal Cats. UFH at 250 U/kg SC every 6 hours produced likely therapeutic anticoagulation in one study[11] In another study, UFH heparin dosage of 300 U/kg SC every 8 hours[35] most consistently provided the plasma concentration associated with greatest clinical efficacy and least hemorrhagic complications in humans. Neither enoxaparin 1 mg/kg SC every 12 hours nor dalteparin 100 U/kg SC every 12 hours induced sustainable anticoagulant activity as measured in vitro.[11] Enoxaparin at 1 mg/kg SC every 12 hours possibly decreased thrombus formation in a venous stasis model.[21]

Thromboprophylaxis in Dogs. The majority of published information regarding heparin use in dogs describes prevention of venous thrombosis in canine IMHA. Prior descriptions of UFH therapy in dogs with IMHA have reported doses of 50-500 U/kg SC.[36,37] In one study, 49% of canine IMHA patients developed clinical signs consistent with thromboembolism (TE), despite treatment with UFH at 100-200 U/kg SC every 8 hours.[38] Two studies reported poor outcome for dogs receiving similar doses.[16,37] Note, however, that in humans, subtherapeutic heparin therapy markedly increases the risk for thrombosis.[39] Plasma heparin monitoring in dogs with IMHA has indicated that most canine IMHA patients required higher doses (up to 560 U/kg) to maintain therapeutic plasma levels.[16] A prospective study describing individual adjustment of heparin dose to target anti-FXa activity of 0.3-0.7 U/mL reported higher survival in comparison with that achieved with other approaches.[16]

Thromboprophylaxis in Cats. Because venous thrombosis is rare in cats, heparins are generally used for thromboprophylaxis in cats at risk for arterial thrombosis or thromboembolism (ATE). No outcome-based studies have evaluated any heparin dose for cats with ATE, and recommendations are highly variable. Most cats receive UFH SC at either 50-100 U/kg ("low-dose") or 200-300 U/kg ("high-dose") every 6-8 hours.[40,41] In cats with ATE there is wide individual variation in heparin pharmacokinetics, with some cats requiring very high doses (up to 475 U/kg) to maintain plasma concentrations within the therapeutic range.[33]

Coumarin Derivatives (Warfarin)

Warfarin has been administered to humans at risk for either venous or arterial thrombosis for decades. Extensive outcome-based studies have defined optimal dosing and monitoring approaches for a variety of specific clinical indications in humans, but little information is available for veterinary patients.

Mechanism of Action. The vitamin K–dependent coagulation proteins (prothrombin, factors VII, IX, and X) and regulatory proteins (protein C and protein S) are synthesized as inactive pro-zymogens. These pro-zymogens are converted to their active forms by the enzyme vitamin K epoxide reductase. Warfarin exerts its anticoagulant effect by inhibiting this enzyme.[5]

Adverse Effects. Potential adverse effects of warfarin include hemorrhage, which may be severe and possibly fatal, skin necrosis (not reported to date in dogs or cats), and teratogenicity. Warfarin is relatively inexpensive, but the costs associated with its use may be high because of the requirement for drug reformulation and frequent international normalized ratio (INR) monitoring.[42]

Monitoring. Prothrombin time is the laboratory test recommended for monitoring warfarin therapy in humans, and it must be adjusted for variations in thromboplastin reagents and laboratory technique using the international sensitivity index (ISI)/INR system. In humans, the recommended therapeutic range for INR depends on the condition predisposing to thrombosis, with target INR up to 3.0.[42] No studies have prospectively evaluated the effectiveness of any warfarin regimen in animals. Because the reagent sensitivity indexes are determined using human plasma, the ISI/INR system may not be valid for animals.

Pharmacokinetics and Pharmacodynamics in Normal Dogs. Early studies suggested a starting dose of 0.22 mg/by mouth (PO) every 12 hours.[43]

Pharmacokinetics and Pharmacodynamics in Normal Cats. Anecdotal reports of use of warfarin in cats at risk for ATE suggest a starting dose of 0.5 mg/cat/d.[44] The pharmacokinetics and pharmacodynamics of warfarin in normal cats indicate an appropriate initial dose of 0.06-0.09 mg/kg/d, although there is marked individual variation and the drug has a narrow therapeutic index.[45,46] Warfarin is highly protein bound (primarily to albumin) in cats,[45] and minor shifts in albumin status or concomitant use of other protein-bound drugs may result in massive changes in the degree of anticoagulation.

Thromboprophylaxis in Dogs. Descriptions of clinical use are limited. A recent retrospective study described an initial dose of 0.05-0.2 mg/kg PO every 24 hours, with dose adjustment to achieve an INR of 2.0-3.0.[47]

Thromboprophylaxis in Cats. Cats at risk for ATE receiving warfarin experience similar or shorter survival times than those receiving

other agents or no thromboprophylaxis. Adverse event rates were high, with up to 1 in 6 experiencing fatal hemorrhage. Given the lack of demonstrable improvement in long-term survival, the need and expense of close monitoring, and the risk of fatal hemorrhage, the use of warfarin in cats is difficult to justify.[40]

Direct Small Molecule Inhibitors

The understanding of enzymatic structure and function has led to the recent development of many small molecules that directly inhibit coagulation enzymes, several of which are approved for use in humans. Rivaroxaban and apixaban are oral FXa inhibitors that have been used in experimental animal models.[48] In vitro evaluation of anticoagulation via addition of rivaroxaban to canine[49] and feline[50] blood has been described in the veterinary literature. Argatroban, a thrombin inhibitor that must be administered via IV infusion, has also been used in experimental canine models.[51]

Platelet Antagonists

In humans, antiplatelet therapy is primarily indicated for conditions associated with arterial thrombosis or thromboembolism, where high shear causes platelet activation. Platelet antagonists are not indicated for prevention of venous thrombosis or pulmonary thromboembolism.

Thromboxane Antagonists

Aspirin (acetylsalicylic acid) is one of the most widely used drugs in human and veterinary medicine because of its antiinflammatory, antipyretic, and antiplatelet properties. Aspirin inhibits cyclooxygenase, reducing the synthesis of prostaglandins such as prostacyclin (PGI_2) from the endothelium, and thromboxane A_2 (TXA_2), produced primarily in platelets and the kidney. PGI_2 is a potent platelet inhibitor, whereas TXA_2 is a potent platelet agonist, so the balance of these 2 prostanoids is important.[52]

Low-dose aspirin (generally 1-2 mg/kg/d) tends to be preferred for long-term antiplatelet therapy in humans because gastrointestinal side effects are minimized, and because lower doses selectively inhibit the production of platelet TXA_2, while sparing endothelial PGI_2 synthesis. The selective inhibition occurs via 2 mechanisms: 1) endothelial cells can produce new cyclooxygenase, whereas anucleate platelets cannot, and 2) low oral dosages expose platelets to aspirin in the portal vasculature, but systemic endothelial exposure is minimal because of high first-pass hepatic metabolism. A large body of evidence indicates that low-dose aspirin therapy in humans reduces or prevents cardiovascular events in patients at risk, and that lack of platelet inhibition ("aspirin resistance") increases the risk of cardiovascular events.[52]

Animal Dosing Protocols.

Dogs. The ideal aspirin protocol for prevention of TE in dogs is not known. Reported data vary regarding the appropriate dose to inhibit canine platelet activity in vivo. Antiplatelet activity was reported using in vitro methods (platelet aggregometry,[53] PFA-100,[54,55] or cyclooxygenase expression[55]) for aspirin administered to healthy dogs at dosages of 8-20 mg/kg, but one report indicated that 10 mg/kg did not affect platelet function when measured by aggregometry.[56] Recent studies evaluating low doses have also reported variable results. Platelet aggregation was impacted in healthy dogs in response to 0.5 mg/kg in some studies,[53,57] but neither platelet-leukocyte interactions[57] nor platelet P-selectin expression[57] were affected. Another report indicated no effect on platelet function (measured by aggregometry or PFA-100) unless dogs received at least 1 mg/kg.[58] Doses of 1 mg/kg/d failed to consistently decrease thromboxane metabolites in the urine, suggesting inadequate in vivo antiplatelet effects of this dose in healthy dogs.[59] No study has reported the impact of antiplatelet therapy on platelet function in dogs with diseases predisposing to TE, where platelets may be hyper-reactive.[60] A single retrospective study reported that aspirin at 0.5 mg/kg/d PO was statistically associated with improved short-term outcome in dogs with IMHA,[37] but the study suffered from major limitations (because of the retrospective nature of the analysis) including temporal bias, unequal treatment group sizes, lack of randomization or blinding, nonstandardized treatment protocols, and minimal patient follow-up. Based solely on the aforementioned retrospective report,[37] aspirin at 0.5 mg/kg/d PO has become a mainstay for thromboprophylaxis in dogs with IMHA.[61] The wide adoption of this approach may be in large part a function of the minimal costs associated with low-dose aspirin therapy, and the ease of use in any clinical setting. In contrast, a different retrospective comparison of survival to 6 months for dogs with IMHA (again, with the inherent limitations of a retrospective analysis) indicated that those treated with aspirin at 0.5 mg/kg/d had poorer survival (40%) as compared with those receiving individually adjusted heparin therapy (88%).[62] A funded, prospective, randomized, double-masked controlled clinical trial is ongoing that will compare outcome in dogs with IMHA receiving low-dose aspirin with those receiving individually adjusted heparin therapy.

Cats. Thromboprophylaxis with aspirin has been commonly recommended for cats at risk for TE at a dose of 81 mg/cat PO every 48-72 hours, but clinical evidence suggests that its efficacy for preventing TE is questionable.[44] Additionally, adverse effect rates are fairly high, with approximately 25% of cats experiencing limiting gastrointestinal signs.[40] One retrospective report of long-term therapy in 24 cats with previous arterial TE showed that aspirin at a dose of 5 mg/cat every 72 hours was associated with similar or lower rates of TE recurrence when compared with other thromboprophylactic therapies, and adverse effects were rare.[40] No controlled studies have evaluated low-dose aspirin in cats. It remains to be determined whether a lower dose approach would be of benefit.

Adenosine diphosphate (ADP) Receptor Antagonists

Ticlopidine, clopidogrel, and prasugrel are thienopyridine derivatives that act in vivo as specific antagonists of $P2Y_{12}$. They are prodrugs with no apparent antiplatelet activity. Metabolic steps that involve cytochrome P450-dependent pathways are required to generate the active metabolites. This need for metabolism causes a significant delay in the onset of antiplatelet effects. The active moiety is a reactive thiol derivative that binds irreversibly to the $P2Y_{12}$ receptor. These metabolites irreversibly and selectively inhibit ADP-dependent platelet aggregation and ADP-induced adenylyl cyclase downregulation. They also cause a dose-dependent reduction of the platelet binding sites for ADP.[63] Thienopyridines inhibit platelet aggregation as triggered by multiple agonists, inhibit shear-induced platelet aggregation, and cause platelet aggregates to be more susceptible to disaggregation. Other pharmacological effects that may contribute to the antithrombotic properties of these drugs include decreased circulating levels of fibrinogen, inhibition of erythrocyte aggregation, stimulation of nitric oxide production, inhibition of expression of tissue factor on endothelial cells, and inhibition of fibronectin synthesis.[63]

Thienopyridines are widely used in humans for the secondary prevention of major vascular events in patients with a history of cerebrovascular, coronary, or peripheral artery disease.[63] Note that all circumstances in which thienopyridines are of proven clinical benefit are in the arterial setting.

Ticlopidine

Pharmacokinetic and Pharmacodynamic Properties. Ticlopidine (Ticlid, Riche Pharmaceuticals, Nutley, NJ) is rapidly absorbed after oral administration. Bioavailability is increased by administration with food and decreased by antacids. The antiplatelet effect takes 2 to

3 days to manifest. In humans, steady state is not achieved until 14 days of repeated dosing, and elderly patients demonstrate a longer half-life and higher area under the curve for the drug.[63]

Dog. Doses of 30-100 mg/kg/d inhibited in vitro platelet aggregation[64,65] and improved patency of vascular grafts in vivo.[65]

Cat. Dosages of up to 100 mg/cat/d failed to consistently alter platelet function. Higher dosages up to 500 mg/cat/d reduced in vitro platelet aggregation and impacted in vivo bleeding times, but caused dose-limiting adverse effects.[66]

Adverse Effects. The most commonly reported adverse effects in humans are nausea, vomiting, and diarrhea (in up to 50% of patients). Rarer and more serious effects include minor or major bleeding (particularly when ticlopidine is combined with other antiplatelet or anticoagulant drugs), bone marrow suppression, thrombotic thrombocytopenic purpura, cholestatic changes, hepatotoxicity, rash, colitis, and arthritis.[63]

Clinical Use.

Human. Large-scale clinical trials have indicated that ticlopidine is effective in prevention of stroke, transient ischemic attacks, and myocardial infarction in a variety of clinical settings.[63]

Dog. Treatment with ticlopidine in dogs that were experimentally infected with heartworms resulted in less severe vascular lesions than observed in untreated dogs.[67] Ticlopidine decreased clot formation and improved dialysis performance when added to heparin in a canine hemodialysis model.[68]

Clopidogrel

Pharmacokinetic and Pharmacodynamic Properties. Clopidogrel (Plavix, Bristol-Myers Squob, New York, NY) is rapidly absorbed after oral administration. Bioavailability is not affected by food or antacids. In humans, approximately 90% of the drug is excreted intact (combination of urinary and fecal excretion). The antiplatelet effect takes 5 days to reach maximum effect.[63]

Dog. A dosage of 2 mg/kg/d failed to consistently impact platelet aggregation in one study, whereas 3 mg/kg/d achieved maximal (42%) platelet inhibition by day 5, and 4 mg/kg/d (50% inhibition) by day 3. If a loading dose of 10 mg/kg was administered, platelet inhibition was achieved with a lower daily dose.[69] Another study indicated adequate platelet inhibition at either 2 or 4 mg/kg/d.[58] Rifampin co-administration (causing activation of the cytochrome P450 system) resulted in greater platelet inhibition at lower doses, and cimetidine co-administration (competitive inhibition of cytochrome P450 system) resulted in less platelet inhibition.[62,70]

Cat. Clopidogrel administered orally to normal cats at 18.75-75 mg/cat/d significantly decreased in vitro platelet aggregation in response to ADP and collagen, and significantly increased oral mucosal bleed time. The maximal effect was reached within 3 days of initiating the drug, and resolved within 7 days of discontinuing the drug. No adverse effects were noted.[71]

Adverse Effects. In general, adverse effects are less severe than those observed with ticlopidine, but similar in nature. They include bleeding, gastrointestinal signs, aplastic anemia, and thrombotic thrombocytopenic purpura in humans. Veterinary Information Network message boards include multiple postings regarding development of nonregenerative anemia in cats with long-term therapy.

Clinical Use.

Human. Among individuals sensitive to clopidogrel, it has proved to be an effective antithrombotic for prevention of arterial thrombi in multiple large-scale clinical trials. Resistance has been described in up to 1 in 5 humans.[72,73] The drug is Food and Drug Administration approved and in wide use for thromboprophylaxis in patients with cerebrovascular disease, coronary artery disease, coronary artery stenting or percutaneous coronary intervention, and peripheral artery disease.

Dog. A small ($n = 12$) case series reported in abstract form comparing clopidogrel (10 mg/kg loading then 2 mg/kg/d) with low-dose aspirin (0.5 mg/kg/d) failed to identify a difference in thrombosis rates or clinical outcome in dogs with IMHA.[74] A larger ($n = 24$) prospective randomized study compared clopidogrel (10 mg/kg loading, then 2 mg/kg/d) with low-dose aspirin (0.5 mg/kg/d) or clopidogrel with aspirin, for prevention of thrombosis in dogs with IMHA. This pilot project also failed to identify any difference in 90-day survival between groups.[75]

Cat. Clopidogrel is in fairly wide clinical use for prevention of arterial thromboembolism in cats at risk, without direct evidence (as yet) supporting efficacy. A multicenter, double-blinded prospective study comparing clopidogrel (18.75 mg/cat/d) with aspirin (81 mg/cat every 3 days) has been ongoing for years, without an indication of superiority for clopidogrel in preventing thrombosis. Enrollment is continuing with the hope that increased patient numbers will provide additional power.

$\alpha_{IIb}\beta_3$ Receptor Antagonists

The platelet glycoprotein receptor IIb/IIIa is an integrin (known as $\alpha_{IIb}\beta_3$) belonging to a family of transmembrane proteins that consist of 2 subunits. These receptors promote cellular adhesion and mediate cell-cell binding. Activation of platelet $\alpha_{IIb}\beta_3$ receptors is the final common pathway leading to platelet activation. Vessel damage, adhesion, and shear forces transform this receptor into a high-affinity binding state that binds von Willebrand factor or fibrinogen, leading to platelet aggregation. These drugs are Food and Drug Administration approved for the prevention of myocardial infarction in humans undergoing percutaneous coronary interventions, with unstable angina, and certain types of acute coronary syndromes.[76] All 3 drugs detailed below are available for intravenous use only and require continuous infusion because of relatively short half-lives. Extensive efforts to develop clinically safe and efficacious oral antagonists of the platelet $\alpha_{IIb}\beta_3$ receptor have not been successful and have been generally abandoned.[77] Clinical use of $\alpha_{IIb}\beta_3$ receptor antagonists has not been described in veterinary medicine.

Abciximab

Abciximab is an anti-integrin Fab fragment of a human-mouse chimeric monoclonal antibody against the human platelet $\alpha_{IIb}\beta_3$ receptor.

Dog. Use of abciximab has been extensively described in canine experimental models of coronary artery stent thrombosis and acute myocardial infarction. Reported doses of 0.2-0.8 mg/kg resulted in $>$ 90% inhibition of platelet aggregation and have been associated with thrombus dissolution.[78]

Cat. Abciximab has also been evaluated in a model of arterial injury in cats. Cats received either aspirin alone, or aspirin and abciximab. Cats in the aspirin and abciximab group showed significantly greater inhibition of platelet function and less thrombus formation than those receiving aspirin alone.[79]

Eptifibatide

Eptifibatide is a cyclic heptapeptide that acts by competitive inhibition because of affinity for the $\alpha_{IIb}\beta_3$ receptor.

Dog. It has been evaluated in experimental studies of canine models of coronary stent thrombosis. The dose protocol of 180 μg/kg every 10 minutes (twice) followed by 2 μg/kg/min constant-rate infusion (CRI) resulted in $>$ 90% inhibition of platelet aggregation. Other

studies have reported doses of 0.5 mg/kg IV followed by 6 μg/kg/min CRI.

Cat. Eptifibatide also inhibits feline platelet aggregation in vitro. However, at doses required to maintain platelet inhibition in normal cats, the drug was associated with idiosyncratic and unpredictable circulatory failure and sudden death.[38]

Tirofiban

Tirofiban is a peptidomimetic nonpeptide (tyrosine derivative) that also competitively inhibits the platelet $\alpha_{IIb}\beta_3$ receptor.

Dog. It has been evaluated in experimental studies of canine models of coronary stent thrombosis. The dose protocol of 180 μg/kg every 10 minutes (twice) followed by 0.3 μg/kg/min CRI resulted in $>$ 90% inhibition of platelet aggregation.[39]

Cat. Use of tirofiban has also been described in an experimental model of cardiac ischemia in cats at a dose of 100 μg/kg IV followed by 5 μg/kg/min CRI. This dose prevented ischemia-induced activation of feline platelets.[80]

Dipyridamole (in combination with aspirin)

Dipyridamole (Persantine and Aggrenox, Boeringer Ingelheim, Germany) inhibits the uptake of adenosine, which inhibits platelet aggregation, causes decreased density of thrombin receptors, inhibits phosphodiesterases causing potentiation of the nitric oxide system, stimulates PGI_2 production, releases tissue plasminogen activator, inhibits smooth muscle proliferation, and has antioxidant and anti-inflammatory properties. Use of dipyridamole improves ischemic tolerance. Although dipyridamole has clear antithrombotic effects in vivo, it has only mild antiplatelet aggregation effects in traditional in vitro studies. It requires very low pH for absorption. It is eliminated by conjugation with glucuronic acid. The antithrombotic benefit of dipyridamole in humans is most evident when administered in conjunction with low-dose aspirin.[81]

Conclusion

Prospective, outcome-based studies are needed to specifically assess the efficacy of appropriately dosed and monitored therapy for prevention of thrombosis and thromboembolism in veterinary patients.

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