INTRODUCTION

The clinical problem

Each year in the United States, an estimated 7 million patients undergo cardiovascular (CV) procedures and operations including percutaneous coronary interventions (PCI), coronary artery bypass graft (CABG) surgery and other heart or vascular surgeries.\textsuperscript{1} Common to all these interventions is that they require an intravenous (IV) infusion of a rapid onset anticoagulant that produces a profound degree of anticoagulation. Heparin effects systemic anticoagulation by inhibiting the activity or generation of most of the procoagulant proteases. Heparin is the anticoagulant of choice for cardiac surgery procedures, due to its rapid onset of action, ability to profoundly inhibit coagulation, short half-life, ease of monitoring and reversibility. However, it has disadvantages as well, such as triggering heparin-induced thrombocytopenia and thrombosis in some patients.\textsuperscript{2} All of the FDA-approved alternatives, such lepirudin, bivalirudin, and argatroban, also have disadvantages of their own. The limitations of all current anticoagulants have prompted efforts to develop novel anticoagulant strategies.

The major limitation of all profound anticoagulant therapy is serious drug-induced bleeding. Therefore, blood usage in cardiac surgery is high, utilizing 10\% to 20\% of the blood supply in the United States.\textsuperscript{3} Thoracic aortic and congenital heart surgeries employing deep hypothermic circulatory arrest (DHCA) require even higher transfusion rates while PCI procedures have reported transfusion rates of 10-15\%.\textsuperscript{4,5}

If bleeding rates are so high, why is such profound anticoagulation used for cardiac surgery procedures? The obvious answer is that thrombosis is far worse for patients than bleeding. The presence of vascular lesions, contact of blood with foreign materials and procedure-induced vascular trauma are strong stimuli for thrombosis. Another significant issue in surgical and non-surgical cardiovascular interventions is the inflammatory response provoked by activation of coagulation and other biological mediators on foreign surfaces such as catheters, intravascular stents or the non-biologic surfaces of the heart-lung machine. The process of diverting blood into a non-physiologic CPB circuit, as well as surgical trauma, ischemia-reperfusion injury and endotoxemia during cardiac surgery also contribute to a profound inflammatory response in the surgical patient.\textsuperscript{6-9} The inflammatory pathways elicited by CPB include complement activation, increased synthesis of pro-inflammatory cytokines, increased leukocyte adhesion and generation of reactive oxygen species, arachidonic acid metabolites, nitric oxide and endothelins.\textsuperscript{10} A number of post-operative complications are attributed to CPB-mediated inflammation, including respiratory failure, renal dysfunction, neurologic dysfunction and multiple organ failure.\textsuperscript{10-13} These post-operative outcomes not only impact on the quality of life of patients but also result in considerable
cost to society.

Much progress has been made in recent years in understanding the sequence of events and pathologic mechanisms that activate hemostatic pathways, including those mediated by tissue factor (TF), fibrinolytic enzymes and platelets. These mechanisms lead to significant thrombin generation. The resulting consumption leads to clotting factor deficiencies, impaired platelet function, thrombocytopenia and systemic fibrinolysis. Profound perturbations of body temperature (rapid cooling and subsequent rewarming) as routinely seen during cardiovascular surgeries employing CPB and/or DHCA further impact hemostasis and impair platelet function. The ongoing nature of the procoagulant (thrombin) and anticoagulant (fibrinolysis) actions in concert with the elevated inflammatory response that occurs during extracorporeal circulation creates opportunities for imbalance that heightens the chance of perioperative thrombosis or bleeding.

Thus, a critical need exists for the development of safer anticoagulant agents, particularly ones whose activity can be readily controlled. Such agents could reduce the number and magnitude of undesired perioperative events such as bleeding and thrombosis. To address this clinical need a number of groups have developed strategies for generating drug-antidote pairs. This strategy utilizes novel anticoagulant aptamers and complementary oligonucleotide antidotes that recognize the primary sequence of the active aptamer. Binding of the antidote causes a change in the three-dimensional conformation of the active aptamer, so that it can no longer bind to its target. Of note, the recent successful clinical translation of an aptamer targeting factor IX (FIX) and its antidote to use in percutaneous coronary interventions demonstrates the feasibility of this strategy. A similar aptamer-antidote approach directed against von Willebrand factor (anti-vWF) has also been developed.

Aptamers and SELEX

Aptamers are generated by in vitro screening of complex nucleic-acid based combinatorial shape libraries (>10^14 shapes per library) employing a process termed SELEX (for Systematic Evolution of Ligands by EXponential Enrichment). The SELEX process consists of iterative rounds of affinity purification and amplification of ligands from combinatorial oligonucleotide libraries to yield high affinity and high specificity antagonists to proteins (Figure 1). Often, the combinatorial libraries employed in SELEX are front-loaded with 2' modified RNA nucleotides (e.g. 2' fluoro-pyrimidines) so that the aptamers generated are highly resistant to nuclease-mediated degradation and amenable to immediate activity screening in cell culture or bodily fluids. Over the past decade this technology has enabled the generation of high affinity and high specificity antagonists to a myriad of proteins including reverse transcriptases, proteases, cell adhesion molecules, infectious viral particles and growth factors (Reviewed in ). In particular, Sullenger et al. have successfully employed this technology to generate potent antagonists of coagulation factors, including human factors VII, VIIa, IX, IXa, X, Xa and thrombin.

As potential therapeutic molecules, aptamers possess a number of useful properties. They are relatively small (8 kDa to 15 kDa) synthetic compounds that possess high affinity and specificity for their target proteins (equilibrium dissociation constants ranging from 0.05-10 nM). Thus, they embody the affinity properties of monoclonal antibodies and soluble single chain Fab fragments made in phage libraries with the chemical properties of small peptides. While initial studies demonstrated the in vitro use of these compounds for studying protein function, more recent studies have demonstrated the utility of these compounds for studying in vivo protein function. In addition, animal and clinical studies to date
have shown that aptamers and compounds of similar composition are well tolerated, exhibit low or no immunogenicity, and are thus likely suitable for repeated administration as therapeutic compounds.\textsuperscript{29,30}

During synthesis, site-specific modifications can be made to rationally alter aptamer bioavailability and clearance. For example, 2’fluoro pyrimidine-modified aptamers in the 10 kDa to 12 kDa size range have a short circulating half-life (~10 minutes) following bolus i.v. administration. Simple chemical modification of the aptamer or conjugation of the aptamer to a high molecular weight inert carrier (e.g. polyethylene glycol or PEG) increases its circulating half-life substantially (6-12 hours).\textsuperscript{31} Finally, because aptamers bind their target proteins by adopting a particular three-dimensional conformation, it is possible to neutralize their activity through the use of complementary oligonucleotides that recognize the primary sequence of the aptamers.

**Summary**

Profound and controllable anticoagulation is essential during a large number of clinical procedures including percutaneous and surgical revascularization procedures (PCI and CABG), aortic surgery and other vascular interventions. Immediate anticoagulation therapy is also required in a variety of thrombotic diseases such as acute coronary syndromes, pulmonary embolism or deep vein thrombosis. Significant risks and side effects of all of these interventions and therapies include profound bleeding or thrombosis leading to cerebrovascular or cardiovascular compromise. The clinical and financial implications of these outcomes can be profound and are associated with prolonged hospitalization, reduced quality of life, and increased health-care resource utilization. Recent basic discoveries of novel therapeutic anticoagulant aptamer/antidote pairs have set the stage for the translation of these agents to clinical practice. Whether this innovative therapeutic strategy can provide superior anticoagulation in prothrombotic environments, while avoiding undesired effects such as bleeding, platelet dysfunction, systemic inflammation and impaired wound healing remains to be determined.

**REFERENCES**


