BRIDGING IN ANTICOAGULATION

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The management of patients on chronic vitamin K antagonist (VKA) therapy who need temporary interruption for an elective invasive or surgical procedure is problematic and complex. Both patient and procedure-oriented risk factors for thrombosis and bleeding have to be individually assessed and properly risk-stratified in the periprocedural period. Certain procedures, such as dental, dermatologic, ophthalmic, and endoscopic procedures can be completed without discontinuing VKA. However, most procedures with a high bleed risk – including major surgeries – will necessitate temporary discontinuation of VKA and consideration of the use of a shorter-acting anticoagulant, usually unfractionated heparin (UFH) or low-molecular-weight-heparin (LMWH), as bridging therapy to maintain functional anticoagulation in patients at intermediate-to-high risk of thromboembolism. Recently completed large, prospective, multicenter cohort studies support the safety and efficacy of mostly therapeutic doses of LMWH in the outpatient setting as periprocedural bridging therapy, provided that post-procedural bleeding risk is taken into account. Large, placebo-controlled, randomized studies are underway by Canadian and US investigators to assess safety and efficacy of various bridging strategies in specific patient populations using LMWH. Lastly, we will review evidence-based periprocedural management strategies for the at-risk patient on chronic VKA, based upon recent narrative and systematic reviews on the subject. The depth and breadth of recent clinical data has necessitated an entire chapter devoted to periprocedural management of antithrombotic therapy for the upcoming 8th ACCP recommendations.

SEVERE SEPSIS

THE PROTEIN C PATHWAY AT THE INTERFACE OF COAGULATION AND INFLAMMATION

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The protein C anticoagulant pathway serves as an on demand natural mechanism to limit thrombotic complications. In addition to being a potent inhibitor of coagulation, the pathway is involved in the regulation of inflammation and cellular death through many mechanisms. Thrombomodulin binds thrombin, thereby accelerating protein C and thrombin activatable fibrinolysis inhibitor (TAFI) activation and at the same time inhibiting thrombin activation of platelets, factors V and VIII and clotting of fibrinogen. The activation of TAFI results in the generation of a carboxypeptidase with a preference for C terminal Arg residues. While TAFI can remove lysine residues from fibrin and slow fibrinolysis, it also removes terminal Arg residues from vasoactive peptides like C5a, thereby protecting the microvasculature from injury. The N terminal lectin domain of thrombomodulin can bind and inhibit HMGB1 and can dampen MAP kinase and NF-κβ signaling. EPCR, a constitutively expressed molecule that binds protein C and factor VII, undergoes internalization and may be a mechanism for regulating the levels of these proteins. Structurally, EPCR is extremely similar to the MHC class 1 family of molecules and has a phospholipid present in the position of the antigen presenting groove of this family, making it extremely likely that it participates directly in immune surveillance. Interestingly, EPCR is a frequent target of antiphospholipid antibodies and the presence of these antibodies is associated with miscarriage. Activated protein C has multiple anti-inflammatory properties. In addition to its anticoagulant activity, it inhibits NF-κβ beta signaling, decreases leukocyte adhesion to endothelium and is cytoprotective in part through inhibition of p53 and Bax and up-regulation of Bcl2. The importance of these properties in disease is becoming apparent. The system is down regulated in atherosclerosis, sepsis, Crohn's disease, diabetes and multiple sclerosis. In most of these disease states, supplementation with activated protein C ameliorates the disease progression, at least in experimental animals. Studies with both antibodies and mutant forms of activated protein C indicate that the anticoagulant and cytoprotective properties of activated protein C can be separated.

THE LIGAND OCCUPANCY OF EPCR SWITCHES THE PAR-1-DEPENDENT SIGNALLING SPECIFICITY OF THROMBIN

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Results from numerous studies, employing endothelial cell-based *in vitro* assays, have indicated that the cleavage of protease activated receptor 1 (PAR-1) by thrombin initiates proinflammatory responses in vascular endothelial cells. Interestingly, the cleavage of the same receptor by activated protein C (APC), bound to endothelial protein C receptor (EPCR), elicits cytoprotective and antiinflammatory responses. The EPCR-dependent PAR-1 cleavage by APC has been hypothesized to account for its beneficial protective effect in severe sepsis. However, noting that thrombin can cleave PAR-1 with ~3-4-orders of magnitude higher catalytic efficiency to elicit proinflammatory responses, it has been difficult to interpret the relevance of the paradoxical PAR-1 cleavage data in the cultured endothelial cells to the *in vivo* settings. In this study, we demonstrate that EPCR is associated with caveolin-1 in lipid rafts of endothelial cells and that the occupancy of EPCR by the Gla-domain of protein C/APC leads to its dissociation from caveolin-1 and recruitment of PAR-1 to a protective signaling pathway through the coupling of PAR-1 to the pertussis toxin sensitive G_i-protein. Thus, when EPCR is bound by protein C, the PAR-1 cleavage-dependent protective signaling responses in endothelial cells can be mediated by both thrombin and APC. Further studies revealed that the cleavage of PAR-4, but not PAR-1, is responsible for the proinflammatory effect of thrombin in cultured endothelial cells that was found to be independent of the occupancy of EPCR by its ligand. Based on these results, we hypothesize that in the presence of protein C the activation of PAR-1 in intact vascular endothelial cells expressing EPCR by both thrombin and APC would elicit only protective responses. These results provide new insight into the mechanism by which PAR-1 and EPCR may participate in protective signaling events in endothelial cells.

ENZYME AND/OR ZYMOGEN?

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Experiments carried out over 20 years ago showed that the infusion of activated protein C (APC) could prevent organ dysfunction and death in baboons challenged with a lethal dose of Escherichia Coli. Because a sublethal dose of E. Coli could be transformed into a lethal one by inhibiting *in vivo* protein C activation, this was taken as evidence that APC was required to counteract the lethal effects of endotoxin. The PROWESS study confirmed this hypothesis, by showing that the infusion of APC (Xigris®) for 4 days was effective in reducing 28- day mortality in patients with severe sepsis, irrespective of the causative infection. However, additional studies in patients with sepsis revealed that APC administration was associated with an increased risk of bleeding, especially in association with low platelet counts and recent surgery. Similar to the ADDRESS study in septic adult patients at low risk of death, the RESOLVE study in pediatric severe sepsis was interrupted for reasons of futility. In this study, there was no difference in overall serious bleeding events during the 28-day study period, but there were numerically more instances of central nervous system bleeding in the Xigris group (4.6%) than in the placebo group (2.1%), particularly in children younger than 60 days. Overall, the results of these studies have had a negative impact on the use of Xigris in clinical practice, even in septic patients at high risk of death. The search for the anti-inflammatory mechanism(s) of APC has resulted in the identification of two pivotal endothelial mediators of the anti-apoptotic and barrier-protective effects of the enzyme: the protein C receptor (EPCR) and protease-activated receptor 1 (PAR1). At variance with PAR1 cleavage by thrombin, PAR1 cleavage by EPCR-bound activated protein C leads to an anti-inflammatory endothelial response. The much higher affinity of thrombin than of activated protein C for PAR1 together with the higher levels of thrombin in severe sepsis represents a major challenge to these *in vitro* results. Two additional findings complicate the issue. While endotoxin-induced disseminated intravascular coagulation and cytokine activation are aggravated in heterozygous protein C-deficient mice, PAR1 (and PAR4) knockout mice all failed to show improved survival or decreased cytokine responses after endotoxin challenge compared with wild type. Second, patients with severe sepsis have a survival advantage, irrespective of Xigris administration, if they are carriers of the factor V Leiden mutation and hence produce more thrombin. Last, but not least, *in vitro* experiments strongly suggest that the protective signaling by APC is mechanistically linked to protein C activation on endothelial cells, with comparable barrier protective effects by exogenous APC requiring a 4-fold higher concentration of the enzyme. Nevertheless, the observation that the anticoagulant activity of activated protein C – responsible for the increased bleeding risk of Xigris - was of minor importance for the survival of animals challenged with endotoxin has elicited the engineering of protein C variants devoid, once activated, of anticoagulant properties, but still retaining anti-inflammatory activity. Zymogens are generally held to exert their relevant functions when activated to enzymes. There are however exceptions to this paradigm; protein S has inherent anticoagulant activity which is independent from activated protein C cofactor function, and both protein S and antithrombin have anti-inflammatory properties, at least *in vitro*, which are more related to ligand occupancy than to their anticoagulant activity. A number of reports suggest a beneficial effect of the infusion of protein C zymogen (Ceprotin®) on the survival of pediatric and adult patients with meningococcal sepsis, a condition apparently associated with a lower endogenous protein C activation capacity. The absence of bleeding complications in these cases is not surprising, given that circulating APC levels are one to two orders of magnitude lower than those detected with the infusion of Xigris. A highly suggestive explanation for the zymogen effect on endothelial cells is the switching of the PAR1-dependent signaling specificity of thrombin from a permeability-enhancing to a barrier-protective response by the ligand occupancy of EPCR.

Intriguingly, the XPRESS trial has shown no difference in 28-day survival of patients with severe sepsis who received Xigris with or without heparin prophylaxis, in spite of the presumably higher rate of APC inactivation with concomitant heparin. A non-inferiority study comparing enzyme and zymogen for the survival of patients with severe sepsis is urgently needed. In the mean time, a rationale exists for the administration of the zymogen in patients presenting with contraindications to APC treatment.

PROPHYLAXIS AND TREATMENT OF THROMBOEMBOLISM II

HOMOCYSTEINE: STILL A RELEVANT THROMBOPHILIA MARKER?

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In the mid 1990s, a meta-analysis of early observational studies on the role of hyperhomocysteinemia as a risk factor for arterial vascular disease found a statistically significant association between homocysteine levels and both coronary heart disease (OR 1.7, 95% CI 1.5-1.9) and stroke (OR 2.5, 95% CI 2.0-3.0). However, most included studies were retrospective, and thus subject to bias and counfounding. Although the magnitude of the association was not confirmed by subsequent pooling of data from prospective cohort studies, an association between hyperhomocysteinemia and cardiovascular disease, especially stroke, was substantially confirmed. Similar findings were reported from retrospective and prospective cohort studies investigating the association between homocysteine levels and venous thromboembolism. Whether homocysteine plays a direct causal role by promoting oxidative stress, endothelial cell damage, inflammation, and thrombosis or it is an innocent bystander remains uncertain. This is particularly true for mild hyperhomocysteinemia (generally defined as homocysteine levels of 15-30 μmol/L), which can be associated to increasing age, smoking, postmenopausal state, metabolic syndrome, sedentary lifestyle, decreased renal function, and use of different drugs. Thus, there is a chance that hyperhomocysteinemia is an epiphenomenon rather than a risk factor. A number of recent studies have investigated the impact of vitamin therapy on homocysteine levels and on cardiovascular events. Vitamin therapy was clearly effective in reducing homocysteine levels, but failed to demonstrate conclusive benefits in the secondary prevention of ischemic heart disease, cardiovascular death, or venous thromboembolism. The results on stroke were more promising, but data appear inconclusive. In all these studies patients were included and treated regardless of their baseline homocysteine levels. The efficacy of vitamin therapy in a more selected population of patients with higher baseline homocysteine levels remains to be demonstrated.

EFFICACY AND SAFETY OF VARIOUS VITAMIN K ANTAGONISTS FOR THE TREATMENT OF SYMPTOMATIC VENOUS THROMBOEMBOLISM

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Introduction. Warfarin is the most widely used vitamin K antagonist (VKA). Although other VKAs are considered to have similar efficacy and safety profiles when targeted to the same INR range, evidence is lacking. We took advantage of two large multinational venous thromboembolism treatment trials to compare efficacy and safety of the different VKAs used. *Methods.* Treatment with a VKA was started within 72 hours and was continued for 3 months. Based