PCI: PROTEIN C INHIBITOR?

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1. HISTORY

1.1. Coagulation

Blood coagulation is a complex chain reaction of enzyme activations in which inactive zymogens are activated by proteolytic cleavage. Each newly activated enzyme in turn cleaves the next zymogen in the chain, resulting in its activation. The final enzyme in the cascade, thrombin, then cleaves soluble fibrinogen into insoluble fibrin which forms a thrombus. A single enzyme can cleave and activate multiple zymogens, thus a small initial stimulus can amplify into a massive wave of coagulation. In addition, thrombin can feedback and stimulate its own synthesis by proteolytically activating two necessary cofactors in thrombin generation, Factor Va and Factor VIIIa. Thus, the coagulation cascade proceeds by both amplification and a feedback positive mechanism, a potentially deadly combination if not closely regulated.

Hemostasis requires a balance of procoagulant and anticoagulant controls. The anticoagulant control mechanisms are both robust and elegant. The first line of defense against blood clotting is the inhibition of thrombin by the serine protease inhibitors (serpins) antithrombin (AT) and heparin cofactor II (HCII) found circulating in the blood. Serpins have reactive site loops that insert into the active site cleft of a serine protease. The protease then cleaves the serpin between the P1–P1' bond, resulting in the formation of a stable covalent intermediate (Travis and Salvesen, 1983). Both AT and HCII act through this common mechanism, blocking thrombin activity by inserting their reactive site loop into the active site of thrombin. The drug heparin stimulates the reaction between thrombin and AT and HCII several thousand-fold. The mechanism is thought to involve the formation of a ternary complex in which heparin binds to heparin binding sites on both thrombin and the serpin, thus forming a “bridge” between the two molecules (Björk and Lindahl, 1982; Rosenberg,
The formation of a ternary complex alone does not fully account for the dramatic increase in activity seen in the presence of heparin. AT goes through a conformational change upon binding heparin which makes it more reactive with thrombin (Olson and Björk, 1992; Rosenberg and Damus, 1973; Shore et al., 1989). Heparin is thought to displace an acidic domain of HCII which can bind to thrombin, thus increasing the rate of inhibition in the presence of heparin (van Deerlin and Tollefsen, 1991). While AT and HCII are important in inhibiting thrombin activity it soon became apparent that some other process was responsible for shutting off thrombin production. This lead to the discovery of the protein C pathway (Esmon, 1992; Kisiel, 1979; Stenflo, 1976).

1.2. Protein C

Once activated, thrombin can proceed down one of two pathways. It can continue to cleave fibrinogen and activate Factor Va and Factor VIIIa, thus stimulating clot formation, or it can bind to thrombomodulin. Thrombomodulin is a membrane bound protein on the surface of endothelial cells. Once thrombin is bound to thrombomodulin it no longer recognizes fibrinogen as a substrate, thus blocking clot formation. Instead thrombin recognizes another inactive zymogen of a serine protease, protein C, as a substrate (Esmon, 1989). Proteolytically activated protein C (APC) requires two cofactors, Factor V and protein S, for full activity (Suzuki et al., 1983). APC specifically cleaves the two cofactors involved in thrombin generation, Factor Va and Factor VIIIa (Kane and Davie, 1988). The destruction of these necessary cofactors results in cessation of thrombin synthesis, and thus stops clot formation. The importance of this pathway in the regulation of coagulation is apparent in the recent discovery that familial thrombophilia is due to a mutation in Factor V which destroys its activity as a cofactor for APC (Dahlback, 1994; Dahlback et al., 1993; Dahlback and Hildebrand, 1994). In this elegant control system thrombin activates a pathway which feeds back and inhibits its own synthesis.

1.3. Protein C Inhibitor

There are several known inhibitors of APC in plasma. α1-Protease inhibitor and α2-macroglobulin are both present in high concentrations in plasma and inhibit APC, albeit at a fairly low rate (Heeb and Griffin, 1988; Hoogendoorn et al., 1991; van der Meer et al., 1989). The presence of a heparin-accelerated inhibitor of APC in plasma was first described by Marlar and Griffith in 1980 (Marlar and Griffith, 1980). Three years later Suzuki et al. purified protein C inhibitor (PCI) from plasma using heparin affinity chromatography (Suzuki et al., 1983). PCI was shown to be a glycoprotein with a molecular weight of 57,000 D. The cDNA for PCI was subsequently obtained in 1987 by screening a human liver library (Suzuki et al., 1987). Sequence analysis showed that PCI belonged to the serpin family, the highest homology being with α1-antichymotrypsin. PCI is a very good inhibitor of APC in vitro. However, the importance of PCI in inhibiting APC was best demonstrated in vivo.

APC was injected into chimpanzees and pre- and post-infusion plasma samples were examined by immunoblotting with anti-protein C antibodies. Complexes between APC and PCI were observed first, and only after PCI had been depleted were complexes between APC and α1-protease inhibitor or α2-macroglobulin observed (Hoogendoorn et al., 1990). In the presence of heparin, complexes between PCI and APC are also formed most readily in human plasma, suggesting that PCI is the primary inhibitor of APC in plasma (Heeb et al., 1989). Complexes between PCI and APC have also been detected in