LECTURE

ΔΙΑΛΕΞΗ

Regulation of coagulation by the antithrombotic protein C pathway

The majority of hereditary thrombophilia defects involves the antithrombotic protein C pathway which includes protein C and protein S as anticoagulant factors, thrombomodulin and endothelial protein C receptor as cofactors for activation of protein C, and factors Va and VIIIa as substrates for activated protein C. Activated protein C (APC) is a normal circulating anticoagulant that is generated from the protein C zymogen by proteolysis. Ischemia induces generation of APC in the brain and heart. APC resistance represents a poor anticoagulant response to APC. Variant factor V containing Gln506 in place of Arg506 (factor V-Leiden) causes APC resistance by impairing the anticoagulant efficiency of the protein C pathway. A variety of lipids and lipoproteins can contribute to either procoagulant or anticoagulant reactions. Interestingly, high density lipoprotein (HDL), "the good cholesterol", is an anticoagulant cofactor for the protein C pathway and studies support the hypothesis that specific minor apolipoproteins and/or lipids of HDL are anticoagulants. Statins, normally used to treat hyperlipidemia, are also remarkably antithrombotics. Anticoagulant lipids include phosphatidyl ethanolamine and cardiolipin. Procoagulant lipids/lipoproteins include triglyceride-rich particles in plasma and oxidized low density lipoprotein (LDL). Procoagulant and anticoagulant lipids/lipoproteins in plasma contribute a Yin-Yang balance that helps influence the up-regulation and down-regulation of thrombin generation. Both diagnostic and therapeutic advances for understanding and treating thrombosis continue to emerge from research on the protein C pathway.

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Ρύθμιση της πήξης μέσω της οδού της αντιθρομβωτικής πρωτεΐνης C

Περίληψη στο τέλος του άρθρου

Key words

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Thrombosis in many patients involves a combination of one or more mild genetic risk factors, often in combination with an acquired risk factor. Because most of the currently known genetic risk factors for venous thrombosis involve defects in the protein C pathway, this review article is focused on the antithrombotic protein C pathway (see 1 and 2 for recent reviews).

1. BLOOD COAGULATION PATHWAYS AND PROTEIN C ANTICOAGULANT PATHWAY

Although more than four decades have elapsed since the first presentation of the cascade model for blood clotting pathways,[3,4] the outline of sequential conversions of protease zymogens to active serine proteases remains useful, with some modifications, to represent blood coagulation reactions (fig. 1). Major advances in the past two decades emphasize both positive and negative feedback reactions (fig. 1).

In positive feedback reactions, alpha-thrombin activates platelets and factors V, VIII, and XI.[5,10] Small amounts of thrombin can be generated by trace amounts of tissue factor via the extrinsic pathway. Subsequently, thrombin can activate factors XI, VIII and V thereby stimulating each of the steps in the intrinsic pathway and thus amplifying thrombin generation. In negative feedback reactions that involve the protein C pathway, binding of alpha-thrombin to thrombomodulin converts the bound thrombin to an anticoagulant enzyme that converts the

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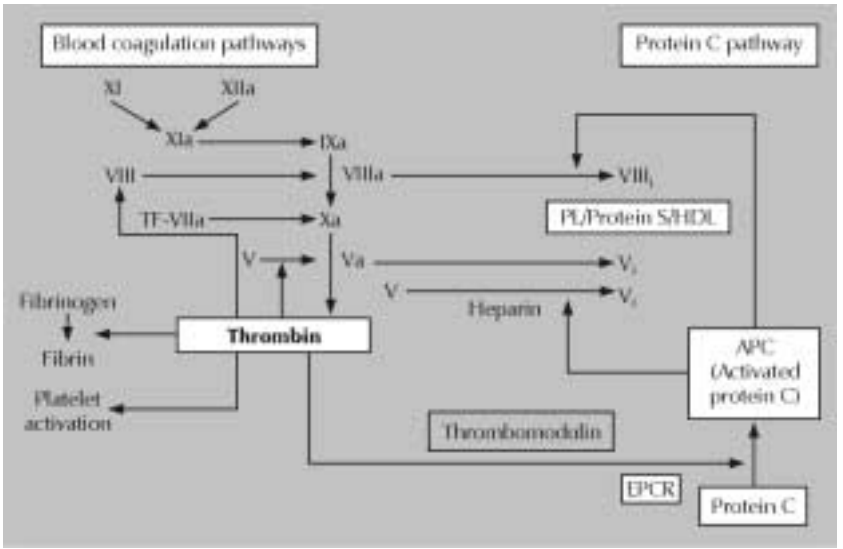

Figure 1. Blood coagulation pathways and anticoagulant protein C pathway. See text for details and discussion.

protein C zymogen to activated protein C (APC), the anticoagulant serine protease (fig. 1). This surface-dependent reaction is enhanced by the endothelial cell protein C receptor (EPCR).[11,13] In a further negative feedback loop, APC with the aid of its nonenzymatic cofactor, protein S, inactivates factors Va and VIIIa by highly selective proteolysis, yielding inactive cofactors, factors V_i and $VIII_i$. Protein S can also directly inhibit factors VIIIa, Xa, and Va.[14,19] Thus, APC and protein S, either in combination or alone, can inhibit multiple steps in the blood clotting pathways. At each step in the coagulation pathways, each clotting protease can be inhibited by one or more protease inhibitors, notably by the heparin-stimulated antithrombin III and tissue factor pathway inhibitor (TFPI).[1] Given the highly nonlinear nature of the coagulation pathways with both positive and negative feedback reactions, synergy between the protein C pathway and plasma protease inhibitors is important for regulating thrombin generation.

2. ACTIVATION OF PROTEIN C

When protein C is synthesized in the liver, a single polypeptide precursor of 461 residues is made with a prepropeptide of 42 amino acids that contains the signal for carboxylation of Glu residues by a carboxylase that forms 9 (-carboxyglutamic acid (Gla) residues and secretion of the mature protein.[20,23] Mature protein C of Mr ~62,000 contains 419 residues and N-linked carbohydrate, and the majority of the secreted protein C zymogen molecules are cleaved by a furin-like endoprotease that releases Lys156-Arg157 and generates a two-chain protein in plasma at 70 nM. The heavy and light chains of plasma protein C are covalently linked by a single disulfide bond that tethers the serine protease globular domain (residues 170–419) to three N-terminal domains, the Gla domain and the epidermal growth factor-like domains, EGF1 and EGF2.[20,24]

Protein C zymogen is activated to a serine protease via cleavage by thrombin at the Arg169-Leu170 peptide bond in a Ca^{2+}-dependent reaction that is accelerated by orders of magnitude by thrombomodulin (see fig. 1).[11] This reaction is also accelerated by another cell surface protein, the endothelial protein C receptor (EPCR).[12,13]

APC in blood likely contributes to antithrombotic surveillance mechanisms because it circulates in all normal humans at 38 pM.[25] The half-life of APC in fresh whole human blood is ~20 min and the *in vivo* half-life of APC in normal adult human subjects is ~22 min.[26,27] The major plasma inhibitors of APC include α_1-antitrypsin, protein C inhibitor, and α_2-macroglobulin.[26]

Perhaps surprisingly, the relationship between thrombin generation and thrombotic tendencies is by no means a positive linear one. In figure 1, thrombin can play central roles for stimulation of both procoagulant and anticoagulant pathways. In experimental animal studies, while it is clear that high levels of infused thrombin are prothrombotic, infusion of low levels of thrombin ironically generate potent antithrombotic activity due to APC generation.[28] The thrombotic potential profile of thrombin in blood is complex and may be represented by a J-curve.[29] This phenomenon has been described as "the thrombin paradox", like the "red wine paradox"–too much of either is harmful to blood vessels, but a little is better than none at all.[29]

Thrombin is indeed a physiologic activator of protein C as shown by experiments in which thrombin infusions into baboons generated anticoagulant activity due to APC.[28,30] Hyperlipidemia and vascular disease can affect protein C activation because it was shown that thrombin infusion into hyperlipidemic monkeys with atherosclerosis generated less APC and caused a poorer *ex vivo* response to APC compared with normolipidemic control monkeys.[31]

3. ISCHEMIA AND PROTEIN C ACTIVATION

Ischemia causes protein C activation *in vivo* because a brief occlusion of the left anterior descending coronary artery in pigs results in APC generation.[32] Furthermore, during cerebral ischemia in patients undergoing routine endarterectomy, circulating APC increases in the venous cerebral blood.[33] Recent studies showed that protein C is significantly activated during cardiopulmonary bypass, mainly during the minutes immediately after aortic unclamping in the ischemic vascular beds.[34] Thus, APC is generated as part of the body's response to injury and to thrombin generation, and a normal anticoagulant response to APC is essential for normal physiology. A poor or subnormal response to APC is identified in many thrombosis patients.

4. RESISTANCE TO ACTIVATED PROTEIN C

The term "activated protein C (APC) resistance", introduced by Dahlback et al,[35,37] indicates a poor anticoagulant response of a subject's plasma to APC. APC resistance is detected in 20–50% of patients with venous thrombosis, depending on criteria for cohort selection.[38,42] Because APC inhibits coagulation by limited proteolysis of factors Va and VIIIa in phospholipid-dependent reactions enhanced by protein S, quite a number of molecular mechanisms are possible. Essentially any genetic or acquired abnormality of a protein C pathway component (fig. 1) that interferes with the expression of APC activity could cause APC resistance, as could an autoantibody against various protein C pathway components. And APC resistance was actually first described in 1990 in patients with venous thrombosis when it was hypothesized to be due to autoantibodies that interfered with expression of APC activity.[43,45]

In spite of the variety of potential causes of APC resistance, the majority of patients with APC resistance, as defined using APTT assays, share the same genetic and molecular defect that involves a point mutation of G to A at nucleotide 1691 in the Factor V gene, predicting the amino acid replacement of Arg506 by Gln.[46,48] Although Gln506-Factor V could equally well be referred to as "factor V La Jolla" or "factor V Amsterdam" since the mutation was simultaneously and independently identified by research groups in these cities, the common usage has evolved in which Gln506-factor V or Q506-Factor V is synonymous with factor V-Leiden. It should be emphasized that the terms "APC resistance" and "factor V-Leiden or Gln506-factor V" are not equivalent because APC resistance is a laboratory phenotype whereas Gln506-factor V is a genotype. This distinction is important, for example, because APC resistance with normal factor V genotype is reported in ischemic stroke patients, venous thrombosis patients and certain subsets of patients with arterial thrombosis.[49,55]

Inactivation of normal Factor Va involves an initial cleavage at Arg506 followed by a phospholipid-dependent cleavage at Arg306.[56,57] Biochemical studies show that Gln506-Factor Va is, indeed, APC resistant as the variant is inactivated ten-times slower than normal Arg506-factor Va.[58,62] Nonetheless, APC can inactivate >90% of the procoagulant activity of Gln506-Factor Va due to cleavage at Arg306.[59] As summarized elsewhere in this volume, factor V-Leiden is a mild risk factor for venous thrombosis. This may be due to the fact that cleavage at Arg306 completely inactivates factor Va or to the possibility that factor Va may be inactivated by proteases other than APC.

5. PROCOAGULANT AND ANTICOAGULANT PLASMA LIPIDS AND LIPOPROTEINS

Based on a variety of clinical studies of atherothrombosis, hypercoagulability is associated with hyperlipidemia and with an increased risk for arterial thrombosis, and treatment of hyperlipidemia lowers risk of thrombosis. However, little consideration is generally given to the potential contributions of hyperlipidemia to venous thrombosis. Yet there are some indirect indications that hyperlipidemia might contribute to increased risk for venous thrombosis. Elevated plasma prothrombin levels associated with the polymorphism 20210A in the prothrombin gene is a mild risk factor for venous thrombosis.[63,64] Increased levels of all vitamin K-dependent procoagulant factors are associated with hypertriglyceridemia, and, furthermore, triglyceride-rich lipoproteins bind vitamin K-dependent clotting factors and promote procoagulant reaction.[65,70] Oxidation of low density lipoprotein (LDL) enhances tenfold its ability to promote prothrombin activation.[71] In contrast to these procoagulant properties of plasma lipoproteins, the "good c-

holesterol", high density lipoprotein (HDL), has anticoagulant cofactor activity for the protein C pathway because HDL enhances factor Va inactivation by APC in the presence of protein S due to cleavage at Arg306 in factor Va.[72] HDL was purified using density gradient centrifugation and HDL2, not HDL3, has anticoagulant cofactor activity for APC (Deguchi, Fernandez and Griffin, unpublished results), showing specific minor apolipoproteins and/or lipids of HDL have anticoagulant cofactor activity.

Blood coagulation reactions are considered to occur at significant rates only on surfaces where anionic phospholipid, especially phosphatidyl serine, is exposed. Recent data support the hypothesis that different lipids differentially enhance either procoagulant or the anticoagulant pathways seen in figure 1. For example, phosphatidylethanolamine and cardiolipin are posited to be, on balance, anticoagulant lipids because they enhance the reactions of the protein C pathway more potently than the procoagulant pathways.[73,74] Consequently, given the large variety of lipid compounds found inside cells, within the membrane and in plasma lipoproteins and given the propensity of almost all clotting factors to associate with lipids that alter the clotting factor's activity, we speculate that defects in anticoagulant lipids will be identified as contributors to increased risk for venous and arterial thrombosis. Moreover, the relationships between plasma HDL and anticoagulant lipid cofactors merits further basic and clinical investigation.

In a recent intriguing report, statins, most often prescribed for treatment of hyperlipidemia, reduced by 50% the incidence of venous thrombosis.[75] If this report is confirmed, there are a number of potential explanations; however, it is tempting to speculate that the reduction in hypercoagulability caused by statins is at least partially due to reduction of procoagulant plasma lipids and lipoproteins.

ΠΕΡΙΛΗΨΗ

Ρύθμιση της πήξης μέσω της οδού της αντιθρομβωτικής πρωτεΐνης C

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Η πλειονότητα των κληρονομικών θρομβοφιλικών διαθέσεων αφορά την οδό της πρωτεΐνης C, η οποία περιλαμβάνει τις πρωτεΐνες C και S ως αντιπηκτικούς παράγοντες (αναστολείς), τη θρομβομοδουλίνη και τους υποδοχείς της ενδοθηλιακής πρωτεΐνης C ως συμπαράγοντες για την ενεργοποίηση της πρωτεΐνης C και τους παράγοντες Va και VIIIa ως υποστρώματα για την ενεργοποιημένη πρωτεΐνη C. Η ενεργοποιημένη πρωτεΐνη C (APC) είναι μια φυσιολογική κυκλοφορούσα αντιπηκτική ουσία, η οποία παράγεται από το προένζυμο (ζυμογόνο) της πρωτεΐνης C με πρωτεόλυση. Η ισχαιμία προκαλεί την παραγωγή APC στον εγκέφαλο και στην καρδιά. Η αντίσταση στην APC συνιστά μειωμένη αντιπηκτική απάντηση στην APC. Η παραλλαγή του παράγοντα V που περιέχει Gln506 στη θέση της Arg506 (παράγοντας V-Leiden) προκαλεί αντίσταση στην APC, διαταράσσοντας την ανασταλτική αποτελεσματικότητα της οδού της πρωτεΐνης C. Ποικίλα λιπίδια και λιποπρωτεΐνες μπορεί να συμβάλλουν είτε στις προάγουσες την πήξη είτε στις αντιπηκτικές (ανασταλτικές) αντιδράσεις. Είναι ενδιαφέρον ότι οι λιποπρωτεΐνες υψηλής πυκνότητας (HDL), η «καλή χοληστερόλη» είναι ένας αντιπηκτικός συμπαράγοντας της οδού της πρωτεΐνης C και μελέτες στηρίζουν την υπόθεση ότι ειδικές ελάσσονες απολιποπρωτεΐνες ή και λιπίδια των HDL είναι αντιπηκτικές. Οι στατίνες που κανονικά χρησιμοποιούνται για θεραπεία της υπερχοληστερολαιμίας, είναι επίσης και αξιόλογα αντιπηκτικά. Αντιπηκτικά λιπίδια είναι η φωσφατιδυλική αιθανολαμίνη και η καρδιολιπίνη. Προπηκτικά λιπίδια/λιποπρωτεΐνες είναι τα πλούσια σε τριγλυκερίδια σωματίδια του πλάσματος και οι οξειδωμένες λιποπρωτεΐνες χαμηλής πυκνότητας (LDL). Τα προπηκτικά και αντιπηκτικά λιπίδια/λιποπρωτεΐνες του πλάσματος συμβάλλουν στην ισορροπία Yin-Yang, η οποία βοηθάει την προς τα άνω και κάτω ρύθμιση της παραγωγής θρομβίνης. Διαγνωστικές και θεραπευτικές πρόοδοι στην κατανόηση και θεραπεία της θρόμβωσης εξακολουθούν να προκύπτουν από την έρευνα της οδού της πρωτεΐνης C.

Λέξεις ευρετηρίου: Θρόμβωση, Λιποπρωτεΐνες υψηλής πυκνότητας, Παράγοντας V, Πρωτεΐνη S

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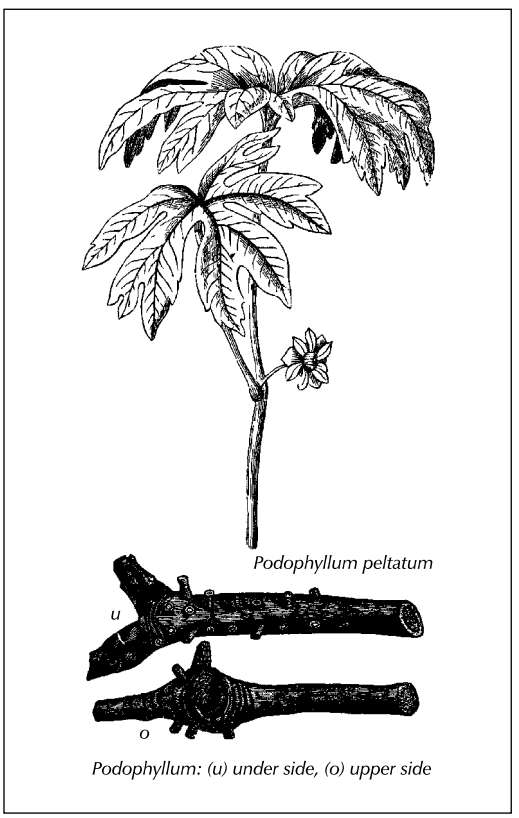

Podophyllum: (u) under side, (o) upper side