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Review

Reciprocal antagonism between inflammation and the protein C system

Antagonism reciproc între inflamație şi sistemul proteinei C

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Abstract

Protein C is a vitamin K-dependent serine protease secreted by the hepatocytes as an inactive zymogen and activated by thrombin bound to endothelial thrombomodulin. An endothelial protein C receptor (EPCR) is involved in both activation and enhancement of protein C activity, resulting in proteolytic degradation of clotting factors Va and VIIIa, thereby providing an efficient anticoagulant mechanism. Evidence was also provided that proinflammatory cytokines would impair the endothelia-mediated activation and activity of the Protein C system by inducing an internalization and proteolytic degradation of thrombomodulin and by shedding EPCR from the surface of endothelial cells membrane. Clinical and experimental studies also emphasized that an inflammatory acute phase reaction is accompanied by a commuted hepatic protein synthesis leading to an increase of plasma fibrinogen, factor VIII:C and of α1 protease inhibitor, while the plasma level of protein C zymogen decrease. On the other hand infusions of activated protein C were reported to protect from a toxico-septic shock by exerting not only anticoagulant but also anti-inflammatory effects.

Keywords: *Protein C, thrombomodulin, endothelial protein C receptor, inflammation-dependent depressive effects, therapeutic approach with activated protein C*

Rezumat

Proteina C, o protează serinică dependentă de vitamina K, sintetizată in hepatocite ca zimogen inactiv, se activează sub acțiunea trombinei în complex cu trombomodulina endotelială. Un receptor endotelial al proteinei C (EPCR) intervine atât în activarea cât şi în optimizarea activității proteinei C activate având ca efect degradarea proteolitică a factorilor coagulării Va şi VIIIa şi exercitând astfel un eficient efect anticoagulant. S-a dovedit că citokinele proinflamatorii perturbă activarea la nivel endotelial inducând internalizarea şi degradarea trombomodulinei şi indepărtând EPCR de la suprafața celulelor endoteliale. Cercetări clinice şi experimentale au demonstrat că reacția de fază acută se asociază cu o comutare a sintezei hepatice de proteine ducând la creşterea fibrinogenemiei, a factorului VIII:C şi a nivelului de α1 inhibitor al proteazelor, în timp ce

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sinteza de zimogen a proteinei C se reduce. Pe de altă parte, infuziile de proteina C activată protejează față de şocul toxico-septic exercitând nu doar efecte anticoagulante, dar şi o activitate antiinflamatorie.

Cuvinte cheie: *proteina C, trombomodulina, receptor endotelial al proteinei C, efecte depresive ale inflamației, abordare terapeutică prin infuzii cu Proteina C activată*

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Inflammations interfere with endothelial-dependent modulation of protein C activation and activity

Anticoagulant Protein C system convincingly illustrates the importance of interaction between circulating plasma proteins and certain receptors and activators located on the surface of endothelial cells. Actually protein C, a vitamin K-dependent serine protease precursor, is secreted by the hepatocytes as an inactive zymogen that will be activated by thrombin bound to endothelial thrombomodulin in the presence of calcium ions. It was demonstrated that the thrombin-thrombomodulin complex may increase the efficacy of protein C activation several thousand times, while thrombin loses its procoagulant effects. The activated protein C (APC) would bind another vitamin K-dependent protein, the protein S, lacking enzymatic activity but acting as a cofactor for protein C. It should be specified that an endothelial protein C receptor (EPCR) is involved in both activation of protein C zymogen and in the enhancement of APC activity. Such activity was found to degrade the activated clotting factors Va and VIIIa proteolitically, thereby arresting coagulation and providing an efficiently sustained antithrombotic effect (1-3).

Evidence was however provided that proinflammatory cytokines-mediated acute phase reaction may impair the functionality of the protein C pathway by inducing enzymes which would degrade the internalized thrombomodulin proteolitically (4) and by shedding EPCR from the surface of endothelial cells membrane, thereby interfering with both activation and activity of protein C (5).

The endothelial (EPCR) receptor removed from the surface of endothelial cells will accumulate in the circulating blood and accordingly its plasma levels were found to be increased in patients with sepsis and also in those with systemic lupus erythematosus (6). Such receptors shed into the circulating blood are inactive functionally and do not potentiate the protein C pathway. Their increased levels may nevertheless provide a reliable marker for detection of disordered vascular endothelial cells.

Acute phase reaction commutes hepatic protein synthesis increasing fibrinogen and decreasing protein C zymogen production

The behavior of plasma levels of activable protein C zymogen or of protein C antigen (PC:Ag) was investigated in leukemic patients and in surgical patients in a critical condition developing an acute phase reaction (7, 8).

Because some patients were affected by sepsis and the increased neutrophil elastase as well as bacterial proteases may have contributed to proteolytic degradation of haemostatic variables (9), it was considered that a more accurate assessment of the inflammatory cytokines-induced changes could be obtained by an experimental aseptic inflammation induced by intramuscular injections of turpentine in rabbits (10). Actually, in agreement with previously mentioned clinical observations (7, 8), experimental study of aseptic inflammation emphasized a decrease of plasma protein C zymogen while plasma fibrinogen levels increased.

It is of note that another acute phase reactant, namely α1 protease inhibitor (α1PI), also known earlier as α1 antitrypsin, was reported to inhibit activated protein C (APC) activity (11) and the hepatic synthesis of this serpin was found to be particularly sensitive to the cytokines

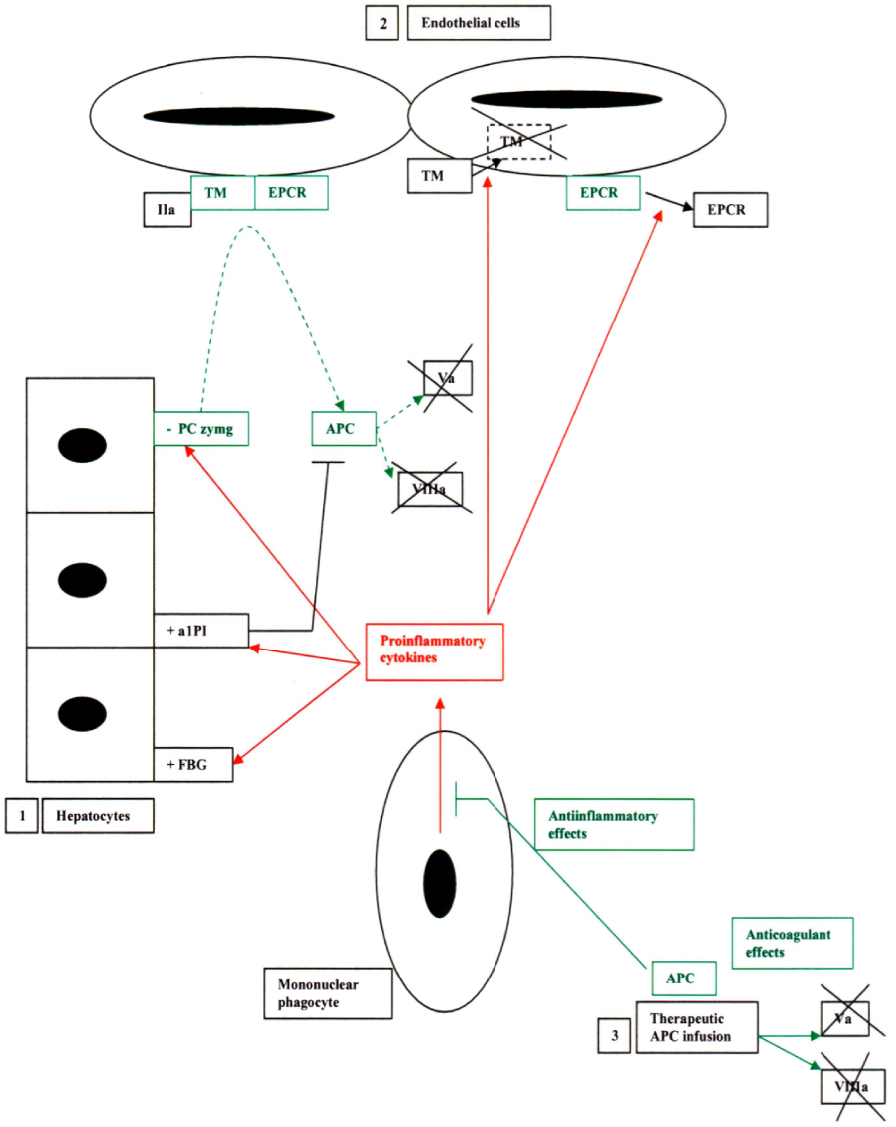

Figure 1. Reciprocal antagonism between anticoagulant protein C system and inflammation:
1. Proinflammatory cytokines released from mononuclear phagocytes reduce the hepatic synthesis of protein C zymogen (PC-zg), while enhancing the production of acute phase reactants such as fibrinogen and the α1 protease inhibitor (α1PI) which also inhibits activated protein C.
2. Proinflammatory cytokines impair endothelia-mediated activation and activity of the protein C pathway by inducing the internalization and proteolytic degradation of thrombomodulin (TM) and by shedding endothelial protein C receptor (EPCR) from the surface of endothelial cell membranes. As a result of reduced hepatic synthesis of protein C zymogen, as well as impaired activation and activity of the protein C system, the anticoagulant effects are diminished and delayed.
3. Sustained infusion of activated protein C (APC) would exert a direct and fast proteolytic degradation of activated coagulation factors Va and VIIIa, thereby arresting the coagulation cascade; APC also inhibits the release of proinflammatory cytokines from the mononuclear phagocytes, thus providing an anti-inflammatory effect beside the anticoagulant one (1,3,6-8,10,14,17).

released from cultured mononuclear macrophage. Actually addition of such macrophage derived cytokines to hepatocytes in culture increased the synthesis of fibrinogen, antithrombin III and of α1PI to 188, 154 and 200% of control values respectively (12).

A reduced hepatic synthesis of protein C zymogen associated with an increased production of fibrinogen and of α1PI is suggesting a cytokine-induced commuted hepatic protein synthesis rather than a generally impaired hepatic protein synthesis. Changes of serum proteins during the acute phase reaction had been previously reported by Werner (13).

As shown in *Figure 1*, inflammatory processes may hinder the protein C system by interfering with its activation at vascular endothelial level, by reducing the hepatic synthesis of protein C zymogen and by inhibiting APC activity. Fortunately in most cases the inhibition of APC by α1PI occurs rather slowly while activation of protein C zymogen and proteolytic degradation of factors Va and VIII proceed much faster, so that the anticoagulant effect would be exerted before the inhibitors-mediated termination of APC activity was achieved.

Sustained infusions with APC may protect from toxico-septic shock

Evidence was provided that infusions of APC could prevent the development of disseminated intravascular coagulation (DIC) and lethality induced by intravenous injections of *Escherichia Coli* cultures in baboons (14). Also the use of protein C concentrate, unfractioned heparin and hemodiafiltration were reported to be beneficial in cases of meningococcus – induced purpura fulminans (15). These beneficial effects appear to be exerted by selectively inhibiting the release of proinflammatory cytokines from human mononuclear phagocytes stimulated with lipopolysccharide (LPS) gamma-interferon (IFN-γ) or phorbol ester (16). It could be specified that binding of activated protein C to a specific receptor on human mononuclear phagocytes inhibits intracellular signaling and monocyte-dependent proliferative responses (17). It should also be mentioned that beside sepsis and major surgery, the development of an acute phase reaction may occur in relation to a sustained use of oral contraceptives (18). It was also claimed that young women displaying high von Willebrand factor and low plasma activity of von Willebrand cleaving enzyme (ADAMTS 13) are at high risk for ischemic stroke and acute myocardial infarction if they are also on oral contraceptives (19).

From a more practical point of view it should be remembered that:

- Increased plasma levels of soluble endothelial protein C receptor (EPCR) could be a reliable marker of a disordered endothelial condition.
- Sustained perfusions with activated protein C appear to be a reasonable approach to the prevention of an impending toxico-septic shock by exerting both anticoagulant and anti-inflammatory effects.

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