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Protein S: A multifunctional anticoagulant

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Abstract

Protein S (PS) is a 75kDa vitamin K-dependent glycoprotein secreted by hepatocytes, megakaryocytes, and endothelial cells. PS is an anticoagulant; functioning as a cofactor to activated protein C (APC) and tissue factor pathway inhibitor (TFPI) as well as acting to directly bind to and inhibit factor IXa (FIXa). PS has other functions including apoptosis, atherosclerosis and as a signaling molecule in the inflammatory pathway through the TAM (Tyro 3, Axl, Mer) family of tyrosine kinase receptors. The importance of PS is manifested by the lethality that could arise when a person has PS deficiency. There are three different types of PS deficiency-each varying in phenotypic lethality-that can lead to thrombotic episodes in a patient. Medication prescribed to treat those who are pro-thrombotic has shown to have its limitations; making the prospect of using PS as an infusion therapy very important. Conversely, with PS playing a role to combat coagulation, using PS antibody as an adjunct hemophilic therapy could be a promising treatment for the future. Understanding PS and its various roles is of great importance to treat patients suffering from pro-thrombotic and hemophilic conditions.

Brief introduction to protein S:

Protein S (PS) is a 75-kDa vitamin K-dependent glycoprotein synthesized by hepatocytes, endothelial cells, and megakaryocytes. Since its discovery in Seattle, Washington in the 1970s, we now know PS to be involved in many physiological processes. PS functions in hemostasis, apoptosis, and inflammation. Curiously, PS is also a ligand for the TAM (Tyro3, Axl and Mer) family of tyrosine kinase receptors, a negative regulator of innate immune response via inhibition of TLR signaling. PS interaction with these receptors regulates inflammation and promotes development of atherosclerosis [1-3]. PS circulates in plasma at a concentration of 350 nM. About 60% of the circulating PS is bound to the C4b-binding protein [1,4]. Unbound Protein S serves as a non-enzymatic cofactor for tissue factor pathway inhibitor (TFPI) and activated protein C (APC) [1,4-6]. Although the functions of PS as a TFPI and APC cofactor have been well established, in 2012, the Majumder laboratory discovered a new function of PS. Acting independent of APC, PS binds and directly inhibits coagulation factor IXa (FIXa) [7].

Anticoagulant function of protein S

The most studied function of PS is as an anticoagulant. PS neutralizes pro-coagulants in three different pathways. The first pathway involves the APC cofactor activity of PS. PS interaction with APC enhances inactivation of factors Va (FVa) and VIIa (FVIIa) [4,8]. In the second pathway, TFPI and PS together enhance creation of the FXa-TFPI complex, which inhibits the TF- factor VIIa complex. Inactivation of FVa and FVIIa along with inhibition of the TF-FVIIa complex ultimately reduces thrombin formation [1,5,6,8]. Finally, in the third pathway, PS inhibits FIXa in the presence and absence of factor VIIa (FVIIa).

Many means have been used to document PS binding and inhibition of FIXa. The Majumder lab showed that addition of PS to normal pooled plasma decreased clotting times. Conversely, supplementation of PS-deficient plasma with increasing concentrations of PS increased clotting times; as expected, addition of anti-PS antibody to these assays caused decreases in clotting times. These results showed that

PS inhibits the intrinsic pathway of the clotting cascade. In addition, the investigators performed modified clotting assays to further prove that the PS inhibitory function was specific to the intrinsic pathway. For example, addition of increasing concentrations of FIX to FIX-deficient plasma caused clotting time to decrease, and anti-PS antibody further shortened clotting times. Conversely, when the investigators supplemented FIX-deficient plasma with FVII instead of FIX, subsequent addition of anti-PS antibody did not change clotting time.

In addition to clotting assays, kinetic studies further confirmed that PS is an inhibitor of FIXa in the intrinsic pathway. The investigators titrated increasing concentrations of PS into PS-deficient plasma that contained a low concentration of tissue factor (TF), and they observed that the amount of thrombin produced decreased as PS concentration increased. The same experiment was repeated with addition of anti-PS antibody, whereupon there was no change in the amount of thrombin generated. As a negative control, the investigators performed this experiment with a high TF concentration, and, again, there was no change in the amount of thrombin generated. This latter control experiment confirmed that the effect of PS on inhibiting thrombin generation was specific to the intrinsic pathway.

Additional experiments were performed to determine whether PS inhibitory effects depended on APC. The investigators used thrombin generation assay (TGA) to measure the amount of thrombin generated in FIX-deficient plasma. The plasma was incubated with low or high TF and FIX, without or with anti-PS antibody, after which the investigators measured peak thrombin generated. The data showed that, with low TF, thrombin generation was low and increased with the addition of anti-PS antibody. However, in the high TF system, thrombin generation did not change [7].

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Protein S and thrombosis

PS deficiency has severe clinical ramifications. PS deficiency has been linked to thrombotic events, such as deep vein thrombosis, pulmonary embolism, and stroke [8-10]. PS deficiency can be inherited or acquired. There are three types of inherited PS deficiency. Type I is characterized by low free and total PS antigen. Type II is characterized by normal amounts of free and total PS, but the activities of both are lower than normal. Normal levels of total PS characterize type III deficiency, but the activity and level of free PS are reduced. Types I and III make up about 95% of all PS deficiencies [8-11]. Mutations in the *PROS1* (PS) gene can be fatal, although individuals with non-lethal mutations have been identified; these individuals have increased risks of thrombotic episodes [1,12-14]. Unbound PS can be measured functionally by performing clotting assays or quantitatively by ELISA [15,16]. ELISA assays are the preferred quantitative method because, unfortunately, clot-based assays have proven to be unreliable and have led to the misdiagnosis of PS deficiency in healthy patients [15,17-20]. Acquired PS deficiency accounts for a small number of cases that result from advanced age, warfarin use, pregnancy, and liver disease [12,13,21-23].

Physicians use blood thinners (anticoagulants) to treat thrombotic/hypercoagulable states. Unfortunately, these therapies can cause excessive bleeding. For patients with comorbidities, it is essential to determine the correct doses of blood thinners; otherwise, individuals may experience severe side effects. Along with elevated bleeding, some anticoagulant therapies have caused platelet activation [21-23]. For example, heparin administration can cause heparin-induced thromboembolism (HIT), requiring health care providers to assess alternative anticoagulant therapies [23]. Many anti-thrombotic medications must be administered multiple times a day, orally or intravenously [16], a requirement that often leads to poor compliance and ineffective therapy.

Targeting protein S in hemophilia

Hemophilia A and B are X-linked bleeding disorders. These disorders are caused by mutation(s) in the FVIII (hemophilia A) and FIX (hemophilia B) genes. An estimated 20,000 individuals in the US have hemophilia, with hemophilia A being four times more prevalent than hemophilia B (<https://www.hemophilia.org>). Individuals affected by these mutations experience mild, moderate, or severe symptoms of the disease. Bleeding occurs because the stability of the platelet plug is compromised [24-29]. Bleeding disorders have been documented since the second century AD, but only recently have we understood the basis of hemophilia. In the 1950s and early 1960s, individuals with hemophilia were treated with only whole blood or fresh plasma. This treatment was inadequate because the blood or plasma did not contain enough pro-coagulant factors. Since then, hemophilia treatment has changed from whole blood transfusions to infusion of recombinant coagulation factors VIII or IX [30]. Unfortunately, patients form inhibitors to factor-driven treatments, mainly in hemophilia A treatment [24,25,27,31-33]. Drawbacks to the current hemophilia treatments have prompted researchers to seek alternatives, such as discovery of longer-acting factors that can be used at lower doses, utilization of non-factor therapies, and gene therapy. Gene therapy particularly is expected to be the key to curing hemophilia [34,35].

Another alternative treatment for hemophilia might be blocking anti-coagulants such as PS, thereby allowing the pro-coagulants within individuals to perform their functions undisturbed. For example, in 2006, Angelillo-Scherrer and coworkers showed that clotting times

were shortened after they injected anti-PS antibody into Hemophilia -ProS-/ mice before tail clipping [31,36].

Conclusion

The importance of PS is evident from the increased risk of thrombosis associated with a PS deficiency, either inherited or acquired. The various functions of PS in hemostasis, inflammation, and atherosclerosis illustrate the biological diversity of PS activities. A key to developing therapies for individuals having elevated thrombotic risks resulting from upregulation of FIXa activity or amount is the fact that FIXa and PS interact directly, in the presence or absence of FVIIa. Protein S could be administered to such individuals to return FIXa activity to the normal range. However, at 75 kDa, PS is somewhat too large to be used efficiently in infusion therapy. Identification of the residues in PS that bind FIXa will provide opportunities to express smaller, functionally active PS derivatives or to synthesize even smaller active PS-derived peptides to use as a therapy. Conversely, designing an inhibitory aptamer against PS could be used to treat hemophilia B because such an inhibitor would permit FIXa to act at full potential.

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