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■ Review

Analysis of Protein C Inhibitor/ SERPINA5

Protein C İnhibitörü/SERPİNA5 analizi

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Abstract

Protein C inhibitor is a non-specific serine protease inhibitor with extensive protease reactivity. Protein C inhibitor (SERPINA5, plasminogen activator inhibitor-3/PAI-3) is a secreted, extracellular clade A serpin. SERPINA5/PCI has originally been described as an inhibitor of activated protein C and independently as an inhibitor of the plasminogen activator urokinase. SERPINA5 binds glycosaminoglycans, phospholipids, and retinoic acid. Glycosaminoglycans and certain phospholipids can modulate its inhibitory activity and specificity. PCI plays role at thrombosis and fibrinolysis, regulation of fertilization, tissue regeneration, vascular permeability, tumor development, invasion, metastasis and angiogenesis. In this review; aimed to provide information about the functions of PCI and to provide guidance for studies.

Key words: Protein C Inhibitor; SERPINA5

Öz

Protein C inhibitörü (PCI), serin proteaz ailesinden (SERPINA5) α1 proteaz inhibitör prototipinde bir plazma glikoproteinidir. İlk olarak insan plazmasında aktive protein C inhibitörü olarak daha sonra ise koagülasyon ve fibrinolizisin inhibitörü olarak tanımlanmıştır. PCI, idrardan sekrete olur ve ürokinaz tipi plazminojen aktivatörü (uPA) ile kompleks oluşturur. Bu nedenle plazminojen aktivatör inhibitörü 3 (PAI-3) olarak da bilinir. PCI'nın tromboz ve fibrinoliziste, fertilizasyonun düzenlenmesinde, doku rejenerasyonunda, vasküler permeabilitede, tümör gelişimi, invazyon, metastaz ve anjiyogenezin düzenlenmesinde rol oynadığı bilinmektedir. Bu derlemede; PCI'nın işlevleri hakkında bilgi vermek ve ileride yapılacak çalışmalar için rehberlik sağlamak amaçlanmıştır.

Anahtar kelimeler: Protein C İnhibitörü; SERPINA5

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Introduction

Protein C inhibitor (PCI) is a plasma glycoprotein in the alpha 1 protease inhibitor prototype from the serine protease family (SERPINA5). It was first described by Marlar and Griffin as an inhibitor of human blood plasma-activated protein C (APC) and later as an inhibitor of coagulation and fibrinolysis. PCI is secretory from the urine (250ng/ml) and complexes with the urokinase-type plasminogen activator (uPA). This is also known as plasminogen activator inhibitor 3 (PAI-3). PCI has heparin binding properties and therefore other protease inhibitors are in the class of heparin-binding serpins such as antithrombin (ATIII) and heparin cofactor II (HCII). Heparin is a negatively charged glycosaminoglycan, secreted by mast cells and a small amount of basophils. Heparin has the task of removing thrombin and complexing with antithrombin III; afterwards at the coagulation cascade of FXa, FXa, FXa and FXa and is in contact with the basic residues of PCI. Heparin does not stimulate PCI inhibition of plasma kallikrein, PSA and HGFA. [1-3] Active Protein C (APC) inhibition can be achieved with 5-10 u / ml heparin. PCI is a good inhibitor of thrombin besides being an APC inhibitor in the hemostatic system; Procoagulant with APC inhibition and anticoagulant with thrombin inhibition. PCI inhibits its target proteases by forming SDS stable 1:1 complexes.

Functions of Protein C Inhibitor

In humans, PCI concentration is in plasma at approximately 4 µg/mL concentration and is thought to originate from the liver. Human PCI is expressed in many organs and tissues, and the protein is present in most body fluids and secretions. PCI expression has been shown in the liver, in the kidney, in the skin, in the heart and in the male and female reproductive tracts [4]

Studies of mice known to be extrinsic to the reproductive organs of the PCI in rodents have revealed PCI expression patterns during mouse development. According to this, it was determined that PCI expression was common in the brain ventricles, in the heart, in the urogenital system, in the skeletal muscles and cartilaginous. [5-8]

The appearance of PCI expression in lung development suggests that PCI may play a role in lung morphogenesis and angiogenesis. The demonstration that PCI plays a role in tissue growth and regeneration by acting as an inhibitor on the activator of the hepatocyte growth factor demonstrates the importance of PCI in cellular processes such as growth signaling.

Protein C Inhibitor at Thrombosis and Hemostasis

Protein C is converted to active protein C (APC) by the binding of thrombin to vascular endothelium. An active protein C resistance (APCR) occurs in the presence of point mutation in the factor V gene. The mutation result is the abnormal Factor V, the factor V Leiden (3). Active protein C resistance is the most common hereditary coagulation defect associated with venous thrombosis. This molecular defect is found in 20-40% of patients with deep vein thrombosis. [9]

Patients with deep vein thrombosis and pulmonary embolism were found to have α -1 antitrypsin with APC and PCI complex, indicating that the regulation of the protein C system under pathological conditions is by PCI.

PCI; In the inhibition of coagulation enzymes, the anticoagulant acts as an inhibitor of APC-Protein C, which is activated by thrombin-thrombomodulin, and in the antifibrinolytic role in the inhibition of urokinase. [10]

Furthermore, a significant increase in plasma concentrations of active SERPINA5 has been described in people with myocardial infarction and is seen as a risk marker for acute coronary events. SERPINA5 is defined in complex with proteases, in patients with active coagulation, and in patients receiving thrombolytic therapy, demonstrating that SERPINA5 reacts in vivo with the proteases of the hemostatic system. However, it is questioned how much it contributes to the regulation of hemostasis. [11]

Protein C Inhibitor in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH), which causes recurrent pulmonary embolisms and heart failure, is a rare disease with a frequency of one millionth. Pulmonary arterial hypertension (PAH) is group 1 pulmonary hypertension (PH), which has nothing to do with pulmonary embolism or chronic thromboembolic pulmonary disease. Whereas chronic thromboembolic pulmonary disease may result in precapillary PH, which is group 4 PH and also called chronic thromboembolic pulmonary hypertension (CTEPH). Interestingly, PAH could be associated with in situ thrombosis in the distal pulmonary arteries. [12]. The excess coagulation defect caused by protein C deficiency is a rare cause of pulmonary embolism. Nishii et al. In a study conducted with PCI transgenic mice, it has been shown that PCI reduces the effect of pulmonary hypertension through thrombin inhibition. [13]

PCI in Tissue Repair and Regeneration

PCI is making tissue repair and regeneration by regulating the hepatocyte growth factor activator. Hepatocyte growth factor

(HGF) plays a critical role in tissue regeneration proliferation, providing stimulation of endothelial and epithelial motility of various cell types. HGF is synthesized in hepatocytes and is in the form of proHGF inactive form. Thrombin is responsible for the activation of proHGFA to HGFA. PCI inhibits HGFA by forming an enzyme-inhibitor complex. The role of PCI in tissue regeneration, repair and wound healing through the inhibition of HGF activity suggests that it may be a potential target for lung regeneration therapy . [14-16]

PCI Role at Fertilization

PCI concentration is in plasma at approximately 4 µg/ml; at urine (250 ng/mL) and at seminal plasma 200 µg/mL. Semen coagulation is achieved by a series of biochemical processes designed to protect and direct spermatozoa during migration, ensuring that the sperm can successfully be rubbed. Thus, semen coagulation promotes fertilization. This mechanism is similar to coagulation and fibrinolysis. There are signs of seminal blood clotting factors and fibrinolytic factors; studies on the role of these factors in shed coagulation continue . [17-18]

In vivo and in vitro fertilization studies have shown that sperm of PCI knock-out mice can not bind to the oocytes of normal female mice and that fertilization can not occur. Histological analysis in PCI knock-out mice reveals that sperm cells undergo malformation and that the normally unrealizable spermatogenesis is associated with unexpected proteolytic activity by the damage of sertoli cells in the male urogenital system. PCI inhibits many enzymes in the seminal plasma, but it still has a role in specific stages of fertilization. In theory, it can be assumed that human fertilization is linked to mutations in the PCI gene. However, Gianotten et al. Found no association between variations in the PCI gene and infertility. In relation to this, Bungum and colleagues have demonstrated that a variation in the 6th exon of the PCI gene results in unexplained loss of fertility after in vitro fertilization in 5 men. [19-20]

PCI Role at Microbiology

SERPINA5 has antimicrobial activity attributed to heparin-binding helical H. It is also known that SERPINC1 (antithrombin) with the heparin binding sequence found in the D-helix exhibits antimicrobial activity. So far, there is no in vivo data supporting the role of SERPINA5 in defense against bacteria. Interestingly, in studies conducted, SERPINA5 has been shown to have a protective role against HIV infection. SERPINA5 was defined at high concentrations in the cervicovaginal fluid of women who remained seronegative despite high-risk exposure, compared to women with

high-risk exposure, HIV-positive women and low-risk HIV exposure. [21]

Non-Protein Ligands of SERPINA5

SERPINA5 binds heparin, certain phospholipids such as phosphatidylserine, oxidized phosphatidylethanolamine, phosphoinositides, and cardiolipin and other glycosaminoglycans. Depending on the target protease binding of glycosaminoglycans and phospholipids can stimulate or suppress the inhibitory activity of serpinA5. In vivo SERPINA5 could bind to glycosaminoglycans on cell surfaces as well as to phospholipids exposed on atherosclerotic plaques on apoptotic and/or activated cells [23], and on microparticles. Another non-protein ligand of SERPINA5 is retinoic acid. Corticosteroid-binding globulin (CBG) and thyroxine-binding protein (TBG) are two non-inhibitory members of the serpin family and act as hormone carriers. According to studies ; binding of different hydrophobic hormones to inhibitory serpins, i.e. to SERPINA5, SERPINC1 (antithrombin), SERPIND1 (heparin cofactor II), and SERPINE1 (PAI). And serpinA5 bound retinoic acid.

Cancer & PCI

It is known that PCI (Serpin A5) plays a protective role against tumor development, tumor invasion and tumor metastasis. The various single nucleotide changes (SNPs) identified in the SERPINA5 gene have been found to be associated with the risk of papillary and follicular thyroid cancer. Expression of SERPINA5 appears to be more benign in uterine cancer, while SERPINA5 is suppressed in more aggressive tumors. Several ex vivo and preclinical studies have been performed to analyze the role of SERPINA5 in tumor cell proliferation, migration, metastasis formation, and tumor angiogenesis, and SERPINA5 does not exhibit malignant behavior in these studies. However, the mechanism of these effects has not yet been met. It appears that not all activities are dependent on protease inhibition activity [24-25]

PCI as a Proteomic Target

When PCI is examined for protein technology; two types of proteomic analysis have been shown to be potential therapeutic targets for multiple sclerosis and biomarker in prostate cancer. Proteins were first identified in multiple sclerosis lesion types; lesions were classified as acute plaque, chronic active plaque and chronic plaque. Despite the fact that many protein functions can not be identified in comparative proteomic analyzes, coagulant tissue factor and protein C inhibitors have been found to be involved in the chronic plaque and have proved to be functional. [29-30]

Genetics of Protein C Inhibitor

PCI / SERPINA5 is a single-chain glycoprotein with a molecular weight of 57 kD. It is known that a region that does not encode in the 5' primer leader structure of human PCI, a 19 amino acid signal peptide, a 387 amino acid mature protein, a termination codon (stop codon), a 839 base pair region that does not encode at the 3' end. The molecule contains 5 glycosylation sites: 3 Asn-X-Ser / Thr sequences, 2 Thr / Ser-X-X-Pro sequences. The human PCI gene is located at the long arm of chromosome 14 32.1 (14q32.13), a weight of 45702 Da, 5 exons, 4 introns. The gene contains various cis elements such as the transcription factor Sp-2 binding site at the 5' end, the activator protein (AP) binding site, the inverted A binding site but not the TATA box and CCAAT box sequences. The PCI amino acid sequences show 93%, 71%, 72%, 62%, 63% and 46% similarity in species among apes, cattle, rabbits, rats, hamsters and chickens respectively. Genetic studies on PCI are very limited and are known to be one mutation related to the subject in the literature. We have described the p.Ser188Asp mutation for the first time in a patient undergoing bone marrow transplantation in the PCI gene. This change is the only mutation in Human Gene Mutation Database (HGMD_CM138902) [33]

Results

Though studies that initially identified the inhibitors of APC and fibrinolysis, and later studies have shown that Protein C inhibitor (PCI), which is known to play a role in many mechanisms ranging from fertilization to cancer, is not only effective in thrombosis and hemostasis, the underlying factors are not fully understood. This deficiency will be overcome with new research on PCI, which is known to have multifunctionality. Table 1 summarises the possible functions of PCI that have been evaluated by various in vitro, ex vivo and in vivo examinations to date.

Declaration of conflict of interest

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Table 1. Possible functions of PCI

Functions of PCI	Target Protease/Cofactor/Proteoglycan
Regulation of protein C pathway	APC/heparin//Thrombin
Regulation of coagulation	Thrombin /Heparin/ Factor Xa, XIa
Regulation of fibrinolysis	u-PA heparin, t-PA heparin
Regulation of sperm capacitation	Acrosin, PSA
Regulation of tumor invasion&metastasis	u-PA
Regulation of angiogenesis	u-PA
Regulation of tissue regeneration	HGFA
Regulation of vascular remodeling	Thrombin
Regulation of APC-mediated anti-inflammation	APC

(APC: Active Protein C, u-PA: Urokinase type plasminogen activator, HGFA: Hepatocyte Growth Factor Activator)

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