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Factor VIIa interaction with endothelial cells and endothelial cell protein C receptor

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Abstract

Plasma coagulation factor VIIa (FVIIa) initiates the coagulation cascade by binding to its cofactor, tissue factor (TF) on cell surfaces, which eventually leads to fibrin deposition and platelet activation. Recent studies showed that FVIIa also binds to endothelial cell protein C receptor (EPCR), a known cellular receptor for anticoagulant protein C/activated protein C, on the endothelium. The present article reviews our current knowledge of FVIIa interaction with EPCR and discusses the potential significance of this interaction in hemostasis, treatment of bleeding disorders with pharmacological doses of FVIIa and FVIIa clearance.

Keywords

Endothelial cell protein C receptor; Factor VIIa; Hemophilia; Protein C

Introduction

The endothelial cell lining constitutes the interface between vascular tissue and the circulating blood, and is positioned optimally to interact with circulating clotting factors. However, thus far very little is known about how various clotting factors interact with endothelium and the consequences of these interactions. In this brief article, we review recent studies on the interaction of factor VIIa (FVIIa) with endothelial cells, particularly FVIIa interaction with endothelial cell protein C receptor (EPCR), and speculate on the potential consequences of this interaction in hemostasis and pathophysiology.

FVIIa interaction with endothelial cells

Studies of FVII binding to intact cells derived from vascular and nonvascular tissues revealed that FVII bound to only nonvascular cells and not to cells derived from vascular tissues, such as aortic endothelial cells [1]. Since native endothelial cells do not express TF, the known cofactor/receptor for FVII/FVIIa, it was not surprising to find the lack of FVII binding to unperturbed endothelial cells. However, Reuning et al. [2] demonstrated FVIIa binding to both stimulated as well as unstimulated human umbilical vein endothelial cells (HUVEC). Since the binding of ¹²⁵I-FVIIa to HUVEC was markedly reduced by 100-fold molar excess of unlabeled prothrombin, factor X and protein C, it was concluded that there were no exclusive, specific binding sites for FVII or FVIIa on the endothelial cells and the FVIIa binding site(s)

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Conflict of interest

The authors state they have no conflict of interest.

was a common binding site for vitamin K-dependent clotting proteins [2]. However, our recent studies showed that the binding of ^{125}I -FVIIa to endothelial cells was blocked by 100-fold molar excess of unlabelled factor X or protein C, but not by other vitamin K-dependent clotting factors, prothrombin or factor IX [3]. These data suggested that the FVIIa binding sites on endothelial cells are not common binding sites for all vitamin K-dependent proteins, but could be shared by protein C and/or factor X. Additional studies revealed that FVIIa associates with endothelial cells through its specific interaction with EPCR [3]. However, FVIIa may also bind to endothelial cells at sites other than EPCR as the EPCR blocking antibodies reduced, but did not completely prevent the association of FVIIa with endothelial cells [3]. Although factor X, similar to protein C, competes with FVIIa to the endothelial cell binding, it is unlikely that FX also binds to EPCR. This is based on the following observations: (i) A 100-fold molar excess of unlabelled factor X failed to inhibit ^{125}I -FVIIa binding to CHO cells transfected to express EPCR [3]; (ii) there is no significant differences in the binding of ^{125}I - factor X to wild-type CHO cells and CHO cells expressing EPCR; (iii) EPCR blocking antibodies had no effect on the binding of ^{125}I -factor X to HUVEC (unpublished data from the authors' laboratories). Nonetheless, Schuepbach and Riewald [4] recently reported that factor Xa also binds to EPCR on cell surfaces. It is unclear at present the reason for the discrepancy between their and our observations. It is conceivable that factor Xa, the ligand used in their studies [4], could interact with cells differently from that of factor X as factor Xa, but not factor X, binds to cell-associated proteins such as TFPI [5] and annexin 2 [6] at the cell surface.

FVIIa interaction with EPCR

EPCR is the cellular receptor for protein C and activated protein C (APC) [7]. It is primarily localized on the endothelial cells of large blood vessels and is very low or absent from the microvascular endothelium of most tissues [8]. Recent studies have shown the expression of EPCR in other cell types, including monocytes [9] and hematopoietic stem cells [10]. EPCR promotes the activation of protein C by thrombin-thrombomodulin complexes [11]. In addition to controlling the coagulation by modulating the protein C-mediated anticoagulant pathway, EPCR has been shown to play a critical role in supporting APC-induced PAR1-mediated cell signaling, which could be responsible for some of the non-hemostatic functions of EPCR and APC [12–16].

Recent studies from our laboratory showed that EPCR also serves as a cellular receptor for FVII/FVIIa on endothelial cells [3]. In parallel studies, Preston et al. [17] demonstrated, using surface plasmon resonance technique, that FVIIa binds to soluble EPCR. These data were further confirmed by Lopez-Sagaseta et al. [18]. Both FVII and FVIIa bind to EPCR with a similar affinity (K_d , ~ 30 to 100 nM range) as that of protein C/APC [3,17,18]. The number of EPCR-specific FVII/FVIIa binding sites on endothelial cells is also very similar to the number of protein C/APC binding sites (~50,000 sites/cell) [3]. These data suggest that EPCR acts a true receptor for FVII/FVIIa.

Studies from Preston et al. [17] revealed that EPCR recognition by protein C is mediated by Leu-8 residue in the ω -loop of the Gla domain. This binding region is completely conserved in human FVII and not in other vitamin K-dependent clotting proteins, which could explain why FVIIa and not other vitamin K-dependent clotting proteins bind to EPCR [3,17]. It is interesting to note that Leu-8 is not conserved in murine protein C but conserved in murine factor VII. In this context, it is interesting to note that human factor Xa, which contains a methionine at this position, was shown to bind to EPCR [4]. It is possible that other cofactors may contribute to factor Xa recognition of EPCR. The same EPCR residues that are directly involved in protein C binding [19] are also involved in the binding of FVII/FVIIa [18], and these residues are completely conserved across human, bovine, rat and mouse [20]. This could explain the ability of mouse EPCR to interact with human protein C [21] and FVIIa [22].

Evidence for FVII/FVIIa interaction with EPCR *in vivo*

FVII circulates in plasma at a 7-fold lower concentration (10 nM) than protein C (70 nM), and thus it is unlikely that FVII acts as the major ligand for EPCR under normal physiological conditions. However, protein C, similar to FVII, binds to EPCR with relatively low affinity (K_d , ~ 30 to 100 nM range) and thus unlikely to occupy all the EPCR sites at its plasma concentration. Under this scenario, the amount of each ligand associated with the EPCR would be approximately proportional to their respective concentrations in plasma. Thus, under normal physiological conditions about 15% of EPCR will be occupied by FVII and the rest of it by protein C. Although direct evidence for FVIIa association with EPCR *in vivo* is lacking at present, a number of recent observations suggest that FVIIa does interact with EPCR *in vivo*. Recent studies have shown that both human and murine FVIIa administered to mice readily associated with the endothelium [22,23]. Further, our preliminary studies (unpublished data of the authors, March 2008) revealed that circulating FVII levels were significantly lower in EPCR overexpressing mice, whereas FVII levels were slightly elevated in EPCR-deficient mice, suggesting that EPCR acts as a binding site for FVII. More importantly, a recent analysis of a large group of healthy individuals showed that individuals with the EPCR Gly variants, whose circulating soluble EPCR levels were higher, had higher levels of FVII and FVIIa, indicating that EPCR *in vivo* serves as a significant reservoir for FVII [24,25].

Potential pathophysiological significance of FVII/FVIIa interaction with EPCR

At present, it is debatable whether FVIIa interaction with EPCR alters FVIIa's coagulant activity. Ghosh et al. [3] showed that FVIIa binding to EPCR on the endothelial cell surface had no influence on FVIIa's coagulant activity, suggesting that EPCR, unlike TF, fails to induce the necessary allosteric conformational changes in FVIIa. However, Lopez-Sagasetta et al. [18] reported a detectable influence of sEPCR on the amidolytic activity of FVIIa, suggesting that FVIIa may undergo a structural rearrangement involving its active site. It had also been reported that sEPCR reduced the ability of FVIIa-sTF to activate factor X, and the EPCR expressed on the endothelial cell surface down-regulated TF-FVIIa activation of factor X at the cell surface [18]. These observations were somewhat surprising as FVII/FVIIa binds to TF with a much higher affinity than it binds to EPCR, and the levels of TF and EPCR on endothelial cells would be very limited. Under these conditions, FVIIa binding to EPCR should not impede FVIIa binding to TF.

APC binding to EPCR has been shown to provide cytoprotective activity through PAR1-mediated cell signaling. There is no evidence at present that EPCR-FVIIa activates PAR1 or PAR2. FVIIa binding to EPCR failed to cleave PAR1 or PAR2 reporter constructs in which alkaline phosphatase was fused to the N-terminus of PARs to monitor the cleavage [3]. Unlike APC, FVIIa failed to activate p44/42 MAPK in CHO cells expressing EPCR [3]. Recent studies of Bae et al. [26] also suggested that FVIIa binding to EPCR does not induce PAR1-mediated cell signaling as it failed to prevent thrombin-induced cell permeability. However, these studies alone do not completely rule out a role for EPCR-FVIIa in cell signaling. Emerging evidence from various groups suggests that the effects of various coagulation proteases on PAR-mediated cell signaling are complex and multifaceted; even undetectable amounts of PAR activation may lead to robust cellular responses [27]. Moreover, the mode of FVIIa-EPCR-induced PAR-mediated cell signaling may differ from that of other proteases. Therefore, further studies are needed to draw a firm conclusion on whether FVIIa binding to EPCR has any relevance for cell signaling.

FVII/FVIIa interaction with EPCR could play an important role in the clearance of FVII/FVIIa from the circulation and/or transport of FVIIa to extravasculature. FVIIa bound to EPCR on endothelial cells was shown to be endocytosed [3]. However, since a majority of the

internalized FVIIa appeared to recycle back to the cell surface [3,22], it is unlikely that EPCR-mediated FVIIa internalization could account fully for the FVIIa clearance. Consistent with this notion, blocking the interaction of FVIIa with EPCR by EPCR blocking antibodies only delayed the clearance of FVIIa, particularly in the early phase, but did not prevent the clearance [22]. Recent *in vitro* and *in vivo* studies suggest that EPCR-mediated endocytosis/recycling could facilitate the transport of FVII/FVIIa from the lumen of blood vessels to the sub-endothelial space [22]. FVIIa administered to mice, immediately following the administration, was found to be associated with the endothelial lining of large blood vessels. Within one hour, FVIIa bound to the endothelium was transferred to the perivascular tissue surrounding the blood vessels and thereafter diffused throughout the tissue. This could facilitate FVII/FVIIa coming into contact with TF in the absence of vascular injury. Trace formation of TF-FVIIa complexes in the sub-endothelial space may prime TF-dependent blood coagulation or propagate basal TF-FVIIa signaling. Matrix associated proteins that are induced by TF-FVIIa signaling [28] may play a role in maintaining vascular integrity [27]. Interestingly, neither FVIIa binding to EPCR nor transport of FVIIa across the endothelium was evident in lung and brain [23], the tissues that are very rich with TF and where thrombin generation could have devastating thrombotic effects. However, recent studies demonstrated EPCR-assisted transport of APC across the mouse blood-brain barrier [29]. It is unclear at present whether real differences exist between EPCR-mediated transport of FVIIa and APC, or if variations in the experimental designs/detection techniques contributed to these differences.

Recombinant FVIIa is widely used for treatment of bleeding episodes in hemophilia patients with inhibitors. The recommended dose of rFVIIa for the treatment varies from 90 to 270 µg/kg [30]. Under these therapeutic conditions, it is conceivable that FVIIa levels in plasma could reach close to protein C levels, resulting in effective competition between protein C and FVIIa for limited EPCR sites on the endothelium. Reduced protein C binding to EPCR in the presence of pharmacological doses of FVIIa would lead to reduced APC generation, as protein C association with EPCR is crucial for its activation by thrombin-thrombomodulin [11]. Experiments performed with cell model systems in which therapeutic concentrations of FVIIa were shown to inhibit thrombin/thrombomodulin-mediated APC generation support this hypothesis [3,18]. Down-regulation of APC generation by FVIIa allows FVa to continue to support factor Xa activation of prothrombin in hemophilia leading to desired thrombin generation. This mechanism may contribute to the lasting hemostatic effect afforded by rFVIIa therapy in hemophilia (Fig. 1). It will be interesting to test the validity of this hypothesis in the future.

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Abbreviations

APC	activated protein C
FVII	factor VII
FVIIa	factor VIIa
EPCR	endothelial cell protein C receptor
HUVEC	human umbilical vein endothelial cells
PAR	protease-activated receptor
s	soluble

TF	tissue factor
TFPI	tissue factor pathway inhibitor

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Fig. 1.

A potential role for EPCR and FVIIa interaction in augmenting the hemostatic effect of rFVIIa in hemophilia. Pharmacological doses of FVIIa can bind weakly to activated platelets and directly activate factor X to factor Xa. Factor Xa upon forming complex with Va on the activated platelet surface catalyzes prothrombin activation to generate thrombin. FVIIa binding to TF expressing cells at the injury site may be necessary and/or contributes to the hemostatic effect by generating initial thrombin that is crucial for activating platelets (this portion of the scheme was adopted from the earlier cell-based model system of coagulation [31]).

Pharmacological doses of FVIIa can also compete with protein C for EPCR and down-regulate the generation of APC. The down-regulation of the protein C/APC anticoagulant pathway by FVIIa would leave factor Va intact for a longer time period, and facilitate thrombin generation in hemophilia.