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Releasing or expression modulating mediator involved in hemostasis by Berythractivase and Jararhagin (SVMPs)

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Abstract

PIII snake venom metalloproteases (SVMPs) are structurally related to ADAMs (a disintegrin and metalloprotease human family of proteins). Berythractivase and Jararhagin are PIII SVMPs with 69% homology with different hemostatic properties. In order to clarify these differences and further characterize the biological effects of these proteins, we compared the effect of both proteases on human umbilical vein endothelial cell (HUVEC) for evaluating the release and modulation of coagulation and fibrinolysis mechanisms as well as the expression of their correlated genes. We found that both proteins increase the von Willebrand factor liberation, but did not modulate gene expression. Berythractivase, differently from Jararhagin increased the expression of tissue factor. Our results showed that both SVMPs (Berythractivase and Jararhagin) activate HUVEC releasing or modulating mediators involved in hemostasis. Meanwhile, we can suggest through the up-regulation of TF gene that the studied SVMP acts in a specific manner, suggesting that Jararhagin has preferentially a local action, while Berythractivase can be assumed as a systemic pro-coagulant protein with activity on the surface of HUVECs.

Keywords: Berythractivase; Coagulation; Endothelial cell; Fibrinolysis; Jararhagin; Prothrombin activator; SVMP

1. Introduction

Snake venoms are rich sources of proteolytic enzymes that affect the blood coagulation system by different ways. The *Bothrops jararaca* and *Bothrops erythromelas* venoms are composed of a complex mixture of metalloproteases, phospholipases A_2 (myotoxins) and serine proteases presenting wide-ranging pathological effects on blood coagulation, cardiovascular system, renal function, fibrinolysis and complement system (Rosenfeld, 1971; Furtado et al., 1991; Ouyang et al., 1992; Fox and Long, 1998). One group of enzymes is usually involved in activating the coagulation cascade, leading to a clot formation. This group includes the thrombin-like enzymes (Selistre and Giglio, 1987; Komori et al., 1993), the prothrombin activator factors, and the factor X activators (Joseph et al., 1999; Silva et al., 2003).

Snake venom metalloproteases (SVMPs) comprise a series of zinc-dependent enzymes, which are relevant components in snake venoms of the Viperidae family (Bjarnason and Fox, 1994). SVMPs are fibrin(ogen)olytic enzymes, acting independent of the plasminogen activation. Incubation of plasma with these enzymes usually promotes incoagulability (Willis and Tu, 1988). They degrade the α- and β-chains of fibrin and fibrinogen and present very little degradation of the γ-chains (Siigur et al., 1998). SVMPs can be divided into four classes

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(P-I to P-IV) according to their domain structure. The P-I class presents only the metalloprotease domain. In the P-II class, a disintegrin domain follows the protease domain. Class P-III enzymes are composed of metalloprotease, disintegrin-like and cysteine-rich domains. The P-IV class has a lectin-like domain in addition to the domains previously described (Jia et al., 1996; Gutierrez and Rucavado, 2000). The catalytic domain shares great functional and structural similarity with the metalloprotease domain of matrix metalloproteases (MMPs) (Blundel, 1994), conserving all the zinc-binding residues and structural characteristics involved in catalysis. In opposition, the disintegrin-like/cysteine-rich domains are found in ADAMs, replacing the hemopexin C domain frequently found in secreted MMPs. The cleavage of proteins at the basement membrane, with the consequent weakening of the capillary structure, constitutes the mechanism by which SVMPs induce hemorrhage (Gutierrez and Rucavado, 2000). Moreover, the catalytic activity of both SVMPs and ADAMs is also related to the shedding of cell surface molecules, thus regulating cell communication and activation (Moura-da-Silva et al., 1996; Black et al., 1997).

The P-III class of SVMPs and ADAMs share homologous metalloprotease, disintegrin, and cysteine-rich domains (Jia et al., 1996). Based on these similarities, the SVMPs have served as early models for ADAMs function. Members of the P-III class have been demonstrating capability for inducing hemorrhage by proteolytic degradation of extracellular matrix proteins, platelet aggregation inhibition and by blocking collagen binding to the $\alpha_2\beta_1$ integrin on platelet surfaces (Fox and Long, 1998; Kamiguti et al., 1996a,b; Kini et al., 2001).

Berythractivase, a P-III class metalloprotease from *B. erythromelas* venom, was previously purified by our group and characterized as a single-chain protein of 78 kDa. Its main biological activity is to promote prothrombin activation (Silva et al., 2003). It belongs to the group A of prothrombin activators (Kini et al., 2001) since the prothrombin activation by Berythractivase is not enhanced in the presence of phospholipids and/or Ca^{2+}. In addition, it also up-regulates endothelial ICAM-1 expression and promotes von Willebrand factor (vWF) releasing from endothelial granules (Silva et al., 2003). Other recent results obtained by our group showed that Berythractivase exerts profound effects on human umbilical vein endothelial cells, as nitric oxide (NO) generation, prostaglandin I_2 (PGI_2) and interleukin-8 (IL-8) releasing (Schattner et al., 2005). In contrast to the majority of PIII metalloproteases, Berythractivase does not show hemorrhagic activity. Its multi-domain structure is in accordance with the standard precursor model of SVMP/disintegrins (Hite et al., 1992).

Another well-known PIII SVMP is Jararhagin from the *B. jararaca* venom characterized as a single-chain protein of 52 kDa (Paine et al., 1992). The strong hemorrhagic activity of Jararhagin is associated to its ability to degrade components of the microvascular basement membrane, to cleave some relevant coagulation proteins (Hati et al., 1999) and to inhibit collagen-induced platelet aggregation (Kamiguti et al., 1996a; Paine et al., 1992). Nevertheless, the alteration of endothelium functional state is another relevant event involved in hemorrhagic disorders. Jararhagin on HUVECs induced NO generation, PGI_2 and IL-8 releasing and decreased cell viability by cell detachment inducing endothelial cell apoptosis (Schattner et al., 2005).

For clarifying the biological effect differences of these proteins, we compared the effect of Berythractivase and Jararhagin on HUVECs evaluating the release and modulation of molecules related to the coagulation and fibrinolysis mechanisms, as well as the expression of their correlated genes. Our results showed that in both of the SVMP studied, there is activation of HUVECs releasing or modulating mediators involved in hemostasis, although in a different manner, suggesting that Jararhagin has preferentially a local action, while Berythractivase presents a systemic action.

2. Materials and methods

2.1. Materials

Berythractivase and Jararhagin were purified as previously described (Silva et al., 2003; Paine et al., 1992). Recombinant Human MMP-3 (Stromelysin-1) was obtained from R&D Systems Inc. (Minneapolis, MN, USA). RPMI 1640 culture medium, HAM F12 culture medium, fetal bovine serum (FBS), trypsin–EDTA, penicillin and streptomycin were purchased from Cultilab (Campinas, SP, Brazil). Collagenase was obtained from Worthington Biochemical Corporation (Lakewood, NJ, USA) and gelatin from ICN Biomedicals Inc. (Aurora, OH, USA). Endothelial cell (EC) growth supplement from bovine neural tissue (ECGS), heparin, L-glutamine, 2-mercaptoethanol, sodium pyruvate, polymyxin B, ethidium bromide, human thrombin and human factor X were obtained from Sigma Chemical Co. (St Louis, MO, USA). Hydrochloric acid, HEPES, $MgCl_2$, NaCl and $CaCl_2$ were obtained from Merck (Darmstadt, Germany). Tumor necrosis factor-alpha (TNF-α) was purchased from BD Biosciences Pharmingen (San Diego, CA, USA). The synthetic peptide substrate of factor Xa Bz-Ile-Glu(γ-OR)-Gly-Arg-pNA (S2222) was obtained from Chromogenix (Mölndal, Sweden). Factor VIIa was purchased from Calbiochem (La Jolla, CA, USA). Enzymeimmunoassay (ELISA) kits for tissue plasminogen activator (t-PA) determination was obtained from Oncogene Research Products (San Diego, CA, USA) and von Willebrand Factor (vWF) was purchased from Diagnostica Stago (Asnieres, FR). TRIzol® Reagent was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Oligo $(dT)_{15}$ primer, dNTP mix, RNasin inhibitor, M-MLV-RT, buffer M-MLV-RT, Taq DNA polymerase, buffer-thermophilic DNA polymerase, 100 or 50 bp Ladder were obtained from Promega Corporation (Madison, WI, USA). The specific primers used for the vWF, t-PA, TF, PAI-1 and GAPDH