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Patagonfibrase modifies protein expression of tissue factor and protein disulfide isomerase in rat skin

María Elisa Peichoto [a], Marcelo Larami Santoro [b, *]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Instituto Nacional de Medicina Tropical, Neuquén y Jujuy s/n, 3370 Puerto Iguazú, Argentina
[b] Laboratório de Fisiopatologia, Instituto Butantan, Av. Vital Brazil, 1500, 05503-900 São Paulo, SP, Brazil

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ABSTRACT

Patagonfibrase is a hemorrhagic metalloproteinase isolated from the venom of the South American rear-fanged snake *Philodryas patagoniensis*, and is an important contributor to local lesions inflicted by this species. The tissue factor (TF)-factor VIIa complex, besides triggering the coagulation cascade, has been demonstrated to be involved in inflammatory events. Our aim was to determine whether patagonfibrase affects the expression of TF and protein disulfide isomerase (PDI), an enzyme that controls TF biological activity, at the site of patagonfibrase injection, and thus if they may play a role in hemostatic and inflammatory events induced by snake venoms. Patagonfibrase (60 μg/kg) was administered s.c. to rats, and after 3 h blood was collected to evaluate hemostasis parameters, and skin fragments close to the site of injection were taken to assess TF and PDI expression. Patagonfibrase did not alter blood cell counts, plasma fibrinogen levels, or levels of TF activity in plasma. However, by semiquantitative Western blotting, patagonfibrase increased TF expression by 2-fold, and decreased PDI expression by 3-fold in skin samples. In agreement, by immunohistochemical analyses, prominent TF expression was observed in the subcutaneous tissue. Thus, patagonfibrase affects the local expression of TF and PDI without inducing any systemic hemostatic disturbance, although that they may be involved in the local inflammatory events induced by hemorrhagic metalloproteinases. Once antivenom therapy is not totally effective to treat the local injury induced by snake venoms, modulation of the activity and expression of TF and/or PDI might become a strategy for treating snake envenomation.

1. Introduction

Tissue factor (TF) is an essential component for initiating blood coagulation *in vivo*. The binding of TF to factor VIIa, in the presence of membrane phospholipids, cleaves factor X and IX, thus initiating blood coagulation. TF is a single-chain integral membrane protein constitutively expressed in vascular smooth muscle cells, adventitial fibroblasts and pericytes, but its expression can be induced on the surface of mononuclear cells, platelets and endothelial cells (van der Poll et al., 2011). Several inflammatory mediators have been shown to promote protein expression and enhanced biological activity of TF (Breitenstein et al., 2010). In addition to express TF molecules on their surfaces, cells have been demonstrated to regulate TF procoagulant activity by means of protein disulfide isomerase (PDI), which regulates the formation of the Cys_{186}-Cys_{209} disulfide bond in the extracellular domain of TF. The oxidation of this disulfide bond renders TF decrypted and with higher procoagulant activity (Kothari et al., 2013; Lysov et al., 2014).

Snake venoms are complex mixtures of proteins, peptides and small organic molecules with a variety of potent enzymatic and ligand-based biological activities (Mackessy and Mackessy, 2009). Among the enzyme-based toxins, an important class includes the snake venom metalloproteinases (SVMP) which act synergistically with many other toxins to induce a complex series of local and systemic pathophysiological effects upon envenomation (Gutiérrez et al., 2009). In a recent publication (Yamashita et al., 2014), increased TF activity in plasma, and increased protein expression of TF in lungs and at the site of inoculation were noticed in rats injected with *Bothrops jararaca* venom. When the crude venom was inhibited by EDTA, TF activity in plasma was drastically reduced, indicating that SVMP were crucial to this increase. Various PIII-

* Corresponding author.
E-mail addresses: mepeichoto@yahoo.com.ar (M.E. Peichoto), marcelo.santoro@butantan.gov.br, marcelosantoro@yahoo.com (M.L. Santoro).

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SVMP (Fox and Serrano, 2008), such as jararhagin from *B. jararaca* venom (Moura-da-Silva and Baldo, 2012), by accumulating on the basement membrane of capillaries and venules, lead to hemorrhage, edema and necrosis, and thus play an important role in local tissue damage. Other PIII-SVMP, such as berythractivase from *Bothrops erythromelas* venom, preferentially exhibit a systemic procoagulant action (prothrombin activation) and are non-hemorrhagic (Baldo et al., 2010; Moura-da-Silva et al., 2008; Silva et al., 2003). Incubation of berythractivase with endothelial cells increases gene and protein expression of TF, whilst jararhagin does not (Pereira et al., 2006). Recently, an activator of factor X and prothrombin isolated from *Bothrops moojeni* (Sartim et al., 2015), moojenactivase, also induced increased procoagulant activity of TF in peripheral blood mononuclear cells.

Patagonfibrase (Pf) is a P-III class metalloproteinase isolated from the venom of the South American rear-fanged snake *Philodryas patagoniensis* (Peichoto et al., 2007, 2010). Local reactions – such as pain, ecchymosis, erythema and edema – are conspicuous signs of snakebites inflicted by this species, but no hemostatic systemic signs, such as hemorrhage or blood incoagulability, are noticed in patients bitten by this snake (de Medeiros et al., 2010). Taking into consideration previous studies (Peichoto et al., 2011) that demonstrated that Pf is an important contributor to local inflammation and local hemorrhage elicited by *P. patagoniensis* envenomation, this study aimed to understand whether altered TF expression induced by hemorrhagic SVMP in dermis could induce *per se* augmented levels of TF in plasma.

2. Materials and methods

2.1. Animals

Male Wistar rats (220–250 g) were obtained from the Animal House of Butantan Institute, and were supplied with free access to food and water. All procedures involving the use of rats were approved by the Animal Ethical Committee of Butantan Institute (protocol 883/12), and were in accordance with the Guide for Care and Use of Laboratory Animals (2011), the International Guiding Principles for Biomedical Research Involving Animals (2012), the Brazilian guidelines from the Conselho Nacional de Controle de Experimentação Animal (CONCEA) (2015), and ARRIVE guidelines. Rats were anesthetized by intraperitoneal administration of xylazine (10 mg/kg b.w.) and ketamine hydrochloride (100 mg/kg b.w.).

2.2. Philodryas patagoniensis venom (PpV) and purification of patagonfibrase (Pf)

A pool of PpV was obtained from wild specimens captured in northeastern Argentina and maintained at the serpentarium of the local Zoo, Corrientes, Argentina. Venom was extracted according to a procedure described previously (Ferlan et al., 1983). Pf was purified from PpV as previously described (Peichoto et al., 2007). Protein concentrations were determined (Smith et al., 1985) using bovine serum albumin (Sigma, USA) as a protein standard.

2.3. Envenomation protocol and sample collection

Pf (60 μg/kg) was administered s.c. to rats; this dose reproduced a characteristic hemorrhagic lesion. Rats injected with saline were used as negative controls. Three hours after Pf injection (period of time considered representative of the acute phase reaction of *Philodryas* envenomation), rats were anesthetized, and blood was collected by puncture of the abdominal aorta. For complete blood counts (CBC), blood (500 μL) was collected into plastic bottles containing 5 μL of 269 mM Na_2-EDTA, and samples were counted in an automated cell counter BC-2800 Vet (Mindray, China). To obtain plasma samples, blood (4.3 mL) was collected into plastic bottles containing 700 μL of CTAD anticoagulant (75 mM trisodium citrate, 42 mM citric acid, 139 mM dextrose, 15 mM theophylline, 3.7 mM adenosine, 0.2 mM dipyridamole, and 2 mM imipramine) (Santoro and Sano-Martins, 2004), and centrifuged at 2500 *g* for 15 min at 4 °C.

One circular 4-cm-diameter skin fragment, whose center was the point of Pf inoculation, was also removed from each animal and used to evaluate protein expression of TF and PDI by Western blotting (WB) (Yamashita et al., 2014), and immunohistochemistry (IH) (Santoro and Sano-Martins, 2004).

2.4. Assays in blood samples

Plasma fibrinogen was assayed as described elsewhere (Ratnoff and Menzie, 1951). Prothrombin time was assayed by incubating plasma samples (80 μL) with rat thromboplastin ((Yamashita et al., 2014), 40 μL) for 1 min at 37 °C, and then 50 mM $CaCl_2$ (40 μL) was added, and clotting times were measured on a Start4 coagulometer (Diagnostica Stago, France). TF activity was evaluated in plasma samples with Actichrome TF kit (American Diagnostica, USA), according to manufacturer's instructions.

2.5. Assays with skin samples

2.5.1. Western blotting

TF and PDI protein expression in rat skin lysate supernatants was evaluated as described previously (Yamashita et al., 2014), except that membranes were incubated at room temperature for 2 h with either 1:500 mouse monoclonal anti-TF antibody (TF9-10H10, Calbiochem, USA) or 1:5000 rabbit polyclonal anti-PDI antibody (Sigma P7372). Expression of β-actin, used as a loading control, was evaluated using 1:5000 mouse monoclonal anti-β-actin antibody (Sigma A5316), and 1:10000 peroxidase-conjugated anti-mouse IgG (Sigma A4416). Membranes were developed as reported elsewhere (Antunes et al., 2010), and densitometric analyses were carried out identically as described (Yamashita et al., 2014).

2.5.2. Histological analysis and immunohistochemistry

Histological sections were stained with hematoxylin-eosin or toluidine blue. TF protein expression in rat skin sections was evaluated as described (Santoro and Sano-Martins, 2004), except that slides were incubated with 1:50 mouse monoclonal anti-TF antibody (TF9-10H10, Calbiochem, USA); negative controls were performed without the use of primary antibody. Specimens were incubated later with 1:100 peroxidase-conjugated anti-mouse IgG (Sigma A4416), and the detection of primary antibody was performed with DAB (3,3′-diaminobenzidine tetrahydrochloride hydrate, Sigma D5637) or AEC (3-amino-9-ethylcarbazole, Sigma A5754) – according to manufacturer's instructions -, and counterstained with 1% neutral red or Mayer's hematoxylin counterstain, respectively.

2.6. Statistical analyses

One-way ANOVA, followed by the Tukey test, was used to compare quantitative results. Statistical analyses were performed using the software SigmaStat (version 3.5, USA). Differences with $p < 0.05$ were considered statistically significant. Data were expressed as mean ± standard deviation (SD).

3. Results and discussion

Subcutaneous injection of Pf did not alter CBC, prothrombin time or plasma fibrinogen levels (Fig. 1a), demonstrating that it did not induce systemic hematologic nor hemostatic alterations, as so does crude *B. jararaca* venom (Yamashita et al., 2014). In addition, normal values of plasma TF activity were observed in samples from Pf-treated animals. This lack of systemic action may be related with the fact that a very low dose of Pf was used, almost 7-fold lower than that used elsewhere (Peichoto et al., 2007), which induced systemic hemorrhage in mice. However, Pf evoked evident local hemorrhage (Fig. 1b), and it increased TF protein expression at this site, in similar levels than that induced by the s.c. administration of PpV (1.6 mg/kg, (Yamashita et al., 2014), data not shown). Three hours after Pf injection, protein bands of 47 and 57 kDa, corresponding to TF and PDI, respectively, were observed, and by semi-quantitative Western blotting, TF expression was noticed to be upregulated two-fold, while PDI expression was downregulated 3-fold (Fig. 1c), similarly to what is observed for *B. jararaca* venom (Yamashita et al., 2014).

The increased plasma TF levels observed during experimental *B. jararaca* envenomation have been ascribed to the action of SVMP, once incubation of crude venom with Na_2-EDTA blocked this phenomenon (Yamashita et al., 2014). Interestingly, Pf increases the expression of TF in skin, but does not lead to increased TF levels in plasma. Pf is a remarkably hemorrhagic PIII-SVMP, similar to jararhagin in action (Peichoto et al., 2007). In fact, hemorrhagic P-III SVMPs, e.g. jararhagin, have been demonstrated to bind collagens I and IV by means of their motif of Da disintegrin subdomain, which grants their accumulation at capillaries and venules close to the site of injection, and in turn restrict their systemic action. On the other hand, berythractivase, which does not possess the same motif, has a systemic activity on coagulation (Baldo et al., 2010; Moura-da-Silva et al., 2008). In line with these findings, our *in vivo* results using Pf evidence that only the local inflammatory reaction induced by hemorrhagic PIII-SVMP is not sufficient to promote the increment in TF levels in circulation. Thus, increased TF levels in plasma during snake envenomation (Yamashita et al., 2014) seems to be due to the release of procoagulating SVMP – e.g. berythractivase or moojenactivase – into the circulation, or to a great extent of the local inflammatory reaction, which would induce TF expression in mononuclear cells derived from circulating blood. In fact, leukocyte-derived microparticles express TF and may bind to activated platelets (Falati et al., 2003).

Similarly to the results exposed in this work, the s.c. injection of *B. jararaca* venom downregulated PDI expression simultaneously to upregulating TF expression (Yamashita et al., 2014). PDI is a fundamental and copious enzyme in the endoplasmic reticulum, essential for catalyzing oxidative protein folding in different cell types (Wang et al., 2015). On the cell surface, PDI has important functions, including the control of TF encryption-decryption and platelet activation, and consequently thrombus formation (reviewed in (Ali Khan and Mutus, 2014; Furie and Flaumenhaft, 2014; Xu et al., 2014)). Besides, PDI has been demonstrated to be an intracellular anti-inflammatory molecule; downregulation of PDI by sepsis significantly increases TNF-α gene expression and release, suggesting that prevention of PDI downregulation might attenuate the inflammatory reaction (Hu et al., 2012; Zhou et al.,

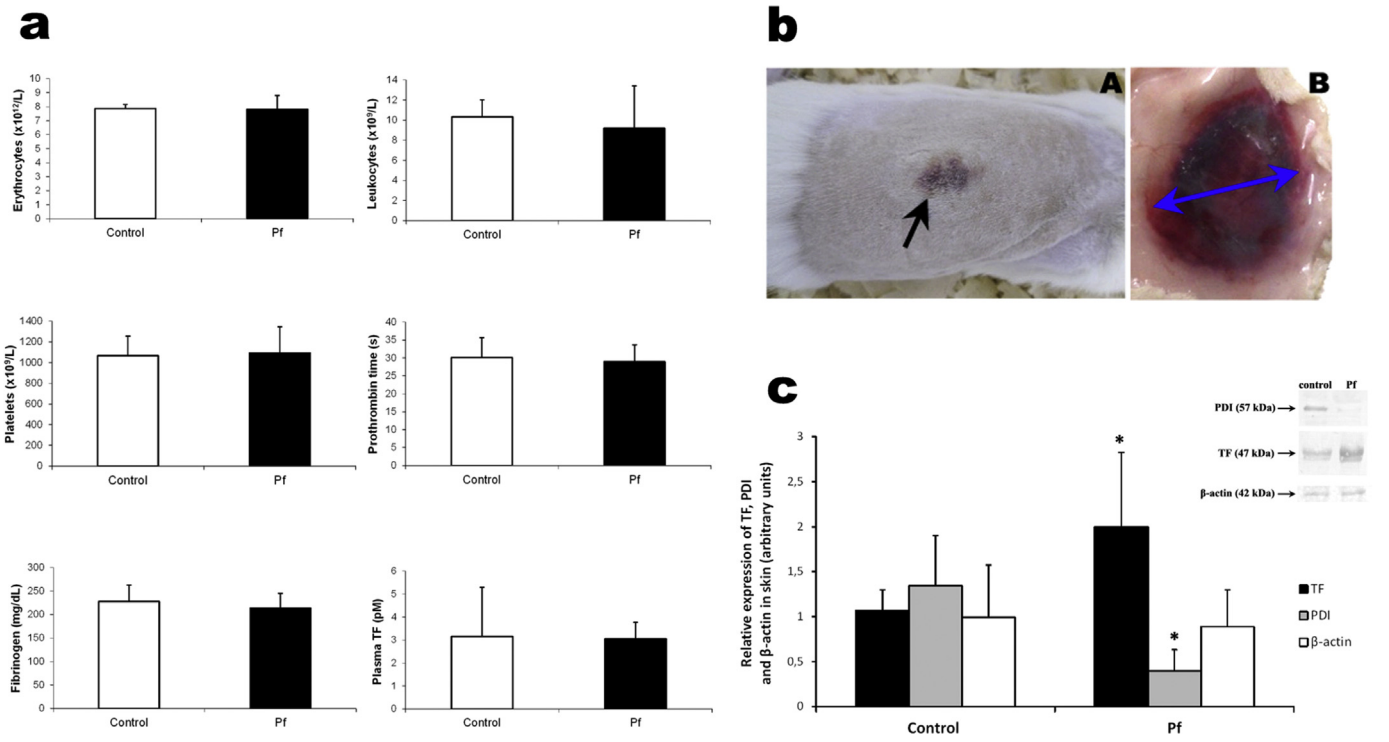

Fig. 1. **a**. Blood cell counts, fibrinogen concentration, prothrombin time, and plasma TF factor levels in blood samples from rats inoculated s.c. with patagonfibrase (Pf, 60 μg/kg) or vehicle (control). The results are expressed as mean ± standard deviation (SD) (n = 4–6 rats/group). **b**. Macroscopic view of dorsal rat skin 3 h after s.c. injection of patagonfibrase (60 μg/kg). **A**- External view of the ecchymosis (black arrow). **B**- The internal hemorrhagic lesion was 4 cm in diameter (blue line). **c**. Western blot detection of tissue factor (TF) and protein disulfide isomerase (PDI) in rat skin lysate supernatants (50 μg of protein) after s.c. injection of patagonfibrase (Pf, 60 μg/kg). Western blot analysis from five individual experiments demonstrated a significant decrease in PDI levels and a significant increase in TF levels. Protein expression was normalized relative to the level of total proteins in each sample. Bars represent the mean ± standard deviation (SD). Asterisks indicate statistically significant differences ($p < 0.05$) with the control group (treated with vehicle). Insert shows representative western blots of TF and PDI proteins in samples of both groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2008). Our results point out that cell injury evoked by Pf down-regulates PDI at the site of inoculation.

We also evaluated the local damage induced by Pf by histology and immunohistochemistry at the site of inoculation. Histologically, hemorrhage and edema were observed concomitant with an inflammatory reaction characterized by the presence of a polymorphonuclear infiltrate (Fig. 2a). With toluidine blue, abundant degranulated mast cells were detected around the blood vessels, mainly below the hypodermis region (Fig. 2b). Immunohistochemical reactions confirmed prominent TF expression in the subcutaneous tissue (Fig. 3), however it is important to note that any kind of cells/microparticles could be explicitly detected herein as the main elements reacting with anti-TF antibodies.

Similarly to the increased expression of TF in dermis induced by Pf, the intraplantar injection of carrageenan, a flogistic agent, induced expression of TF, TNF-α, interleukin (IL) 6 and IL1-β, at the site of injection, mainly by endothelial cells and infiltrating neutrophils and monocytes. However, unlike Pf, carrageenan also induced a systemic inflammatory response (demonstrated by raised levels of fibrinogen and C-reactive protein), acute lung inflammation, and increased immunostaining for TNF-α, IL1-β, and TF in rat lungs (Vazquez et al., 2015). Moreover, increased plasma

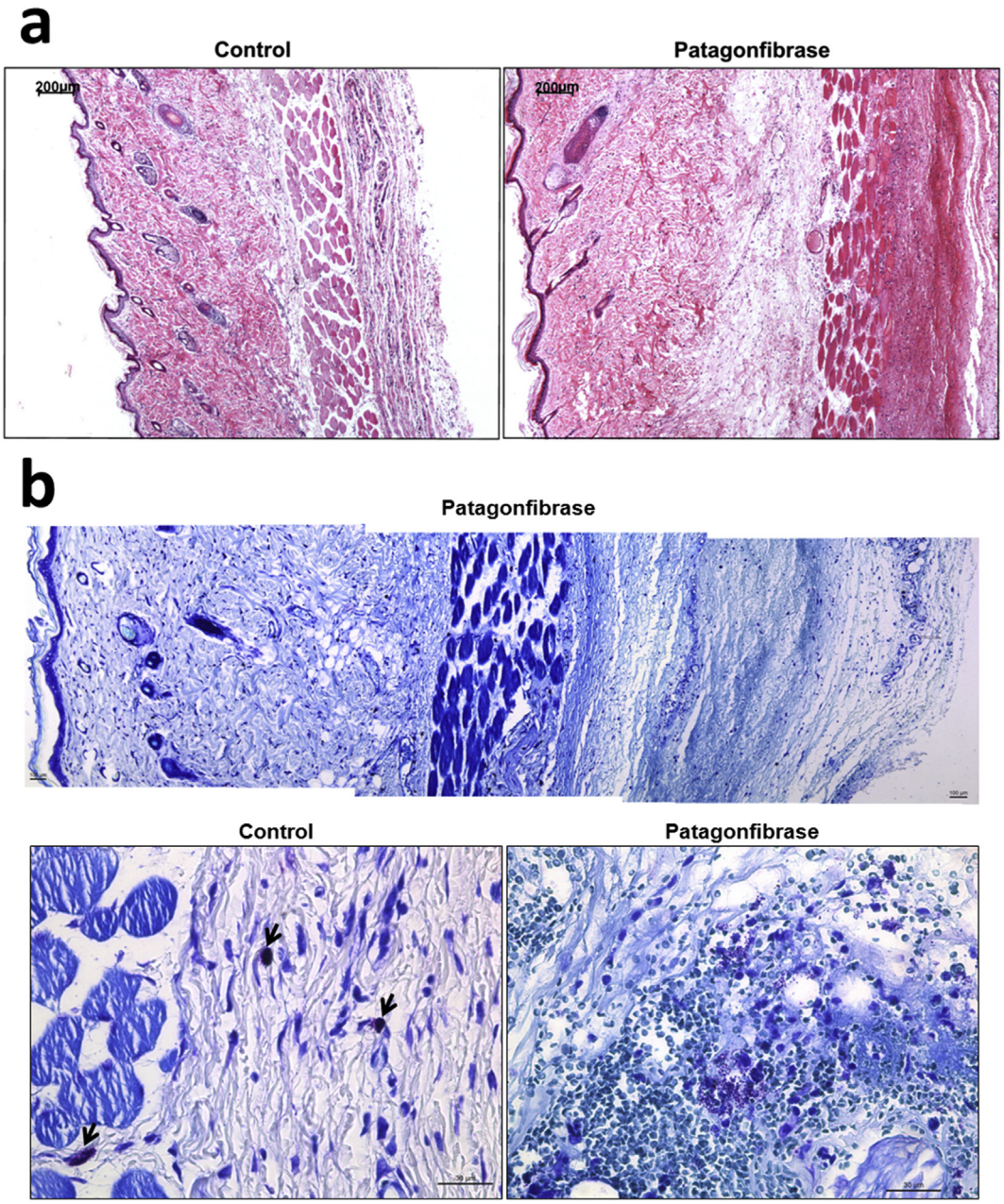

Fig. 2. Light micrographs showing the histopathological changes in rat skin at 3 h after s.c. injection of patagonfibrase (60 µg/kg). **a**. Note: congestion of blood vessels, edema, inflammatory infiltrate of polymorphonuclear leukocytes, and extravasation of erythrocytes induced by patagonfibrase, mainly in and below the hypodermis region. Sections were stained with hematoxylin and eosin. **b**. Note mast cells stained metachromatically with toluidine blue (black arrows) in the subcutaneous tissue of control samples, but they are degranulated in samples treated with patagonfibrase. Sections were stained with toluidine blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

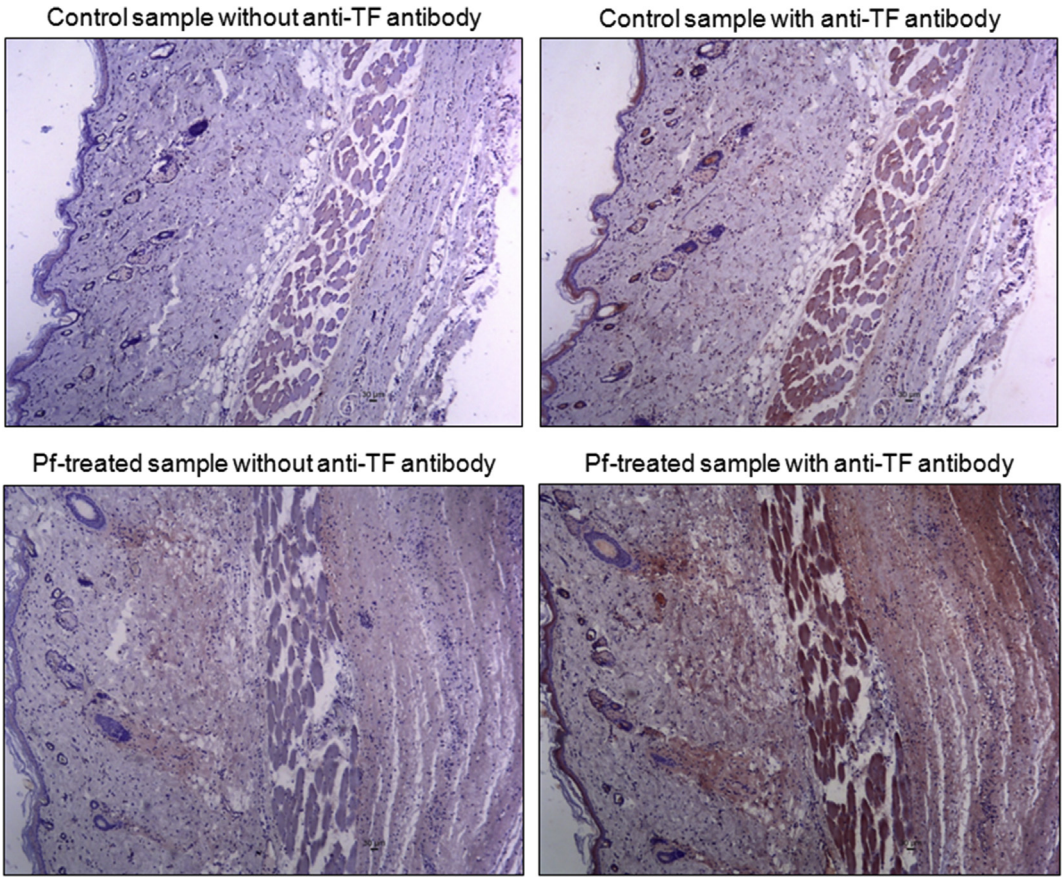

Fig. 3. Immunohistochemistry (peroxidase method with AEC staining) for tissue factor (TF) in rat skin 3 h after s.c. injection of patagonfibrase (Pf, 60 μg/kg). Note the prominent TF expression (labeled in red) induced by Pf in the subcutaneous tissue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels of TF have been associated with the inflammatory condition of sickle cell disease and endotoxemia (Chantrathammachart et al., 2012; Pawlinski et al., 2004).

4. Conclusions

Patagonfibrase effectively increased TF and reduced PDI locally in a rat model of acute hemorrhage without evidencing any systemic action. The agreement of the results from this study with those noticed for *B. jararaca* snake venom in the same model (Yamashita et al., 2014) indicates that the upregulation of TF and downregulation of PDI may contribute to the local inflammatory reactions that characterize snakebites. In fact, besides its hemostatic activity, the cytoplasmic domain of TF has been implicated in the regulation of the immunoinflammatory responses (Sharma et al., 2004). Therefore, taking into account that the antivenom therapy is not very effective locally, modulation of TF or PDI could become a tempting strategy for the treatment of local injuries elicited by snakebites.

Author contributions

M.E. Peichoto and M.L. Santoro conceived, designed and performed the experiments, and analyzed the data; M.L. Santoro contributed reagents/materials/analysis tools; M. E. Peichoto wrote the paper, and M.L. Santoro carefully revised the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Ali Khan, H., Mutus, B., 2014. Protein disulfide isomerase a multifunctional protein with multiple physiological roles. Front. Chem. 2, 70.

Antunes, T.C., Yamashita, K.M., Barbaro, K.C., Saiki, M., Santoro, M.L., 2010. Comparative analysis of newborn and adult Bothrops jararaca snake venoms. Toxicon 56, 1443–1458.

Baldo, C., Jamora, C., Yamanouye, N., Zorn, T.M., Moura-da-Silva, A.M., 2010. Mechanisms of vascular damage by hemorrhagic snake venom

metalloproteinases: tissue distribution and in situ hydrolysis. PLoS Negl. Trop. Dis. 4, e727.
Breitenstein, A., Camici, G.G., Tanner, F.C., 2010. Tissue factor: beyond coagulation in the cardiovascular system. Clin. Sci. (Lond) 118, 159–172.
Chantrathammachart, P., Mackman, N., Sparkenbaugh, E., Wang, J.G., Parise, L.V., Kirchhofer, D., Key, N.S., Pawlinski, R., 2012. Tissue factor promotes activation of coagulation and inflammation in a mouse model of sickle cell disease. Blood 120, 636–646.
de Medeiros, C.R., Hess, P.L., Nicoleti, A.F., Sueiro, L.R., Duarte, M.R., de Almeida-Santos, S.M., Franca, F.O., 2010. Bites by the colubrid snake Philodryas patagoniensis: a clinical and epidemiological study of 297 cases. Toxicon 56, 1018–1024.
Falati, S., Liu, Q., Gross, P., Merrill-Skoloff, G., Chou, J., Vandendries, E., Celi, A., Croce, K., Furie, B.C., Furie, B., 2003. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. J. Exp. Med. 197, 1585–1598.
Ferlan, I., Ferlan, A., King, T., Russell, F.E., 1983. Preliminary studies on the venom of the colubrid snake Rhabdophis subminatus (red-necked keelback). Toxicon 21, 570–574.
Fox, J.W., Serrano, S.M., 2008. Insights into and speculations about snake venom metalloproteinase (SVMP) synthesis, folding and disulfide bond formation and their contribution to venom complexity. FEBS J. 275, 3016–3030.
Furie, B., Flaumenhaft, R., 2014. Thiol isomerases in thrombus formation. Circ. Res. 114, 1162–1173.
Gutiérrez, J.M., Rucavado, A., Escalante, T., 2009. Snake venom metalloproteinases. Biological roles and participation in the pathophysiological of envenomation. In: Mackessy, S.P. (Ed.), Handbook of Venoms and Toxins of Reptiles. CRC Press/Taylor & Francis Group, Boca Raton, pp. 115–138.
Hu, J.Y., Li, C.L., Wang, Y.W., 2012. Altered proteomic pattern in platelets of rats with sepsis. Blood Cells Mol. Dis. 48, 30–35.
Kothari, H., Pendurthi, U.R., Rao, L.V., 2013. Analysis of tissue factor expression in various cell model systems: cryptic vs. active. J. Thromb. Haemost. 11, 1353–1363.
Lysov, Z., Swystun, L.L., Kuruvilla, S., Arnold, A., Liaw, P.C., 2014. Lung cancer chemotherapy agents increase procoagulant activity via protein disulfide isomerase-dependent tissue factor decryption. Blood Coagul. Fibrinolysis 26, 36–45.
Mackessy, S.P., 2009. The field of reptile toxinology. Snakes, lizards, and their venoms. In: Mackessy, S.P. (Ed.), Handbook of Venoms and Toxins of Reptiles. CRC Press/Taylor & Francis Group, Boca Raton, pp. 1–21.
Moura-da-Silva, A.M., Baldo, C., 2012. Jararhagin, a hemorrhagic snake venom metalloproteinase from Bothrops jararaca. Toxicon 60, 280–289.
Moura-da-Silva, A.M., Ramos, O.H., Baldo, C., Niland, S., Hansen, U., Ventura, J.S., Furlan, S., Butera, D., Della-Casa, M.S., Tanjoni, I., Clissa, P.B., Fernandes, I., Chudzinski-Tavassi, A.M., Eble, J.A., 2008. Collagen binding is a key factor for the hemorrhagic activity of snake venom metalloproteinases. Biochimie 90, 484–492.
Pawlinski, R., Pedersen, B., Schabbauer, G., Tencati, M., Holscher, T., Boisvert, W., Andrade-Gordon, P., Frank, R.D., Mackman, N., 2004. Role of tissue factor and protease-activated receptors in a mouse model of endotoxemia. Blood 103, 1342–1347.
Peichoto, M.E., Teibler, P., Mackessy, S.P., Leiva, L., Acosta, O., Goncalves, L.R., Tanaka-Azevedo, A.M., Santoro, M.L., 2007. Purification and characterization of patagonfibrase, a metalloproteinase showing alpha-fibrinogenolytic and hemorrhagic activities, from Philodryas patagoniensis snake venom. Biochim. Biophys. Acta 1770, 810–819.
Peichoto, M.E., Leme, A.F., Pauletti, B.A., Batista, I.C., Mackessy, S.P., Acosta, O., Santoro, M.L., 2010. Autolysis at the disintegrin domain of patagonfibrase, a metalloproteinase from Philodryas patagoniensis (Patagonia Green Racer; Dipsadidae) venom. Biochim. Biophys. Acta 1804, 1937–1942.
Peichoto, M.E., Zychar, B.C., Tavares, F.L., 2011. de Camargo Goncalves LR, Acosta O, Santoro ML. Inflammatory effects of patagonfibrase, a metalloproteinase from Philodryas patagoniensis (Patagonia Green Racer; Dipsadidae) venom. Exp. Biol. Med. (Maywood) 236, 1166–1172.
Pereira, A.L., Fritzen, M., Faria, F., Motta, G., Chudzinski-Tavassi, A.M., 2006. Releasing or expression modulating mediator involved in hemostasis by berythractivase and jararhagin (SVMPs). Toxicon 47, 788–796.
Ratnoff, O.D., Menzie, C., 1951. A new method for the determination of fibrinogen in small samples of plasma. J. Lab. Clin. Med. 37, 216–320.
Santoro, M.L., Sano-Martins, I.S., 2004. Platelet dysfunction during Bothrops jararaca snake envenomation in rabbits. Thromb. Haemost. 92, 369–383.
Sartim, M.A., Costa, T.R., Laure, H.J., Espindola, M.S., Frantz, F.G., Sorgi, C.A., Cintra, A.C., Arantes, E.C., Faccioli, L.H., Rosa, J.C., Sampaio, S.V., 2015. Moojenactivase, a novel pro-coagulant PIIId metalloprotease isolated from Bothrops moojeni snake venom, activates coagulation factors II and X and induces tissue factor up-regulation in leukocytes. Arch. Toxicol. 90, 1261–1278.
Sharma, L., Melis, E., Hickey, M.J., Clyne, C.D., Erlich, J., Khachigian, L.M., Davenport, P., Morand, E., Carmeliet, P., Tipping, P.G., 2004. The cytoplasmic domain of tissue factor contributes to leukocyte recruitment and death in endotoxemia. Am. J. Pathol. 165, 331–340.
Silva, M.B., Schattner, M., Ramos, C.R., Junqueira-de-Azevedo, I.L., Guarnieri, M.C., Lazzari, M.A., Sampaio, C.A., Pozner, R.G., Ventura, J.S., Ho, P.L., Chudzinski-Tavassi, A.M., 2003. A prothrombin activator from Bothrops erythromelas (jararaca-da-seca) snake venom: characterization and molecular cloning. Biochem. J. 369, 129–139.
Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85.
van der Poll, T., de Boer, J.D., Levi, M., 2011. The effect of inflammation on coagulation and vice versa. Curr. Opin. Infect. Dis. 24, 273–278.
Vazquez, E., Navarro, M., Salazar, Y., Crespo, G., Bruges, G., Osorio, C., Tortorici, V., Vanegas, H., Lopez, M., 2015. Systemic changes following carrageenan-induced paw inflammation in rats. Inflamm. Res. 64, 333–342.
Wang, L., Wang, X., Wang, C.C., 2015. Protein disulfide-isomerase, a folding catalyst and a redox-regulated chaperone. Free Radic. Biol. Med. 83, 305–313.
Xu, S., Sankar, S., Neamati, N., 2014. Protein disulfide isomerase: a promising target for cancer therapy. Drug Discov. today 19, 222–240.
Yamashita, K.M., Alves, A.F., Barbaro, K.C., Santoro, M.L., 2014. Bothrops jararaca venom metalloproteinases are essential for coagulopathy and increase plasma tissue factor levels during envenomation. PLoS Negl. Trop. Dis. 8, e2814.
Zhou, M., Jacob, A., Ho, N., Miksa, M., Wu, R., Maitra, S.R., Wang, P., 2008. Down-regulation of protein disulfide isomerase in sepsis and its role in tumor necrosis factor-alpha release. Crit. Care 12, R100.