UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso de Farmácia

Lonomia obliqua venom activity upon extracellular matrix

Alessandra Selinger Magnusson

Porto Alegre, dezembro de 2013.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso de Farmácia

Lonomia obliqua venom activity upon extracellular matrix

Prof. Dr. Marcelo Lazzaron Lamers

Alessandra Selinger Magnusson

Porto Alegre, dezembro de 2013.

Sumário

Agradecimentos

Primeiramente, gostaria de agradecer aos meus pais, Marli e Luiz, os quais me proporcionaram orientação e apoio incondicional para concluir está graduação. Além, de outros objetivos e sonhos.

As minhas irmãs, Luciana e Sara, que nossas diferenças continuem as nós completar e nosso amor a nos unir.

As minhas avós Lúcia e Venância, ensinaram-me que com garra não há dificuldade que a vida apresente que não possa ser superada com amor. Juntamente com minha tia Cecília, que me mostrou a beleza da dedicação e bondade a todos a sua volta. Sei que todas velam por mim.

As minhas tias, Marlene, Ivonete, Isete, Hilda, Marina, Bete e Herminia, que contribuíram cada uma a sua forma e exemplo para com a minha formação pessoal e intelectual.

A Andreia, Rebeca e Bianca que as amizades são inestimáveis. Apoiaram-me em momentos difíceis transformando lágrimas em boas risada/ gargalhadas.

Ao Gustavo por todo apoio, amizade e crescimento durante parte dessa jornada. Por me mostrar que sou capaz de conseguir tudo que desejo.

A Chris Krebs sendo uma das únicas pessoas que compreendeu e continua compreender as dificuldades de trabalhar e cursar Farmácias ambos na UFRGS. Obrigado pelo ombro amigo.

Aos bons corações da Patologia Bucal da UFRGS, docentes e discentes, local onde colegas se tornaram amigos.

Ao meu Orientador, Marcelo, por toda a paciência e confiança.

Apresentação

Este trabalho de conclusão de curso será apresentado sob forma de artigo científico, sendo elaborado segundo as normas da revista científica “Toxicon” (fator de impacto segundo o *Journal Citation Reports* - 2012 = 2,924) conforme documentação apresentada no anexo 1.

Artigo Cientifico

Lonomia obliqua venom activity upon extracellular matrix

Alessandra Selinger Magnusson[a], Renata Maria Soares Terra[a], Antônio Frederico Michel Pinto[a], Jorge Almeida Guimarães[a], Marcelo Lazzaron Lamers[b]

[a] Laboratório de Bioquímica Farmacológica, Centro de Biotecnologia, Federal University of Rio Grande do Sul (UFRGS),

[b] Departament of Morphological Science, Institute of Basic Health Sciences, UFRGS – Porto Alegre, RS, Brazil,

Corresponding author:

Marcelo Lazzaron Lamers, PhD

Department of Morphological Sciences, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul

Rua Sarmento Leite, 500, Porto Alegre, RS, Brazil, CEP 90050-170

e-mail: marcelo.lamers@ufrgs.br

Phone: +55-51-33085011

Abstract

Lonomia obliqua is a medically important caterpillar endemic in South Brazil. Contact with theis caterpillar's bristles causes an envenomation syndrome characterized by ecchymosis and hemorrhage. The *Lonomia obliqua* bristle extract (LOBE) is mainly composed by highly active proteases known to interfere with the hemostatic system of the victims. Although the effects of the venom enzymes upon blood coagulation and platelet aggregation is well characterized, it is possible that LOBE might also affect the environment surrounding small blood vessels, favoring hemorrhage and impairing wound healing. In this work we evaluated the proteolytic activity of LOBE upon extracellular matrix (ECM) components. Degradation of extracellular matrix proteins was performed by *in vitro* digestion of purified proteins (collagen, laminin, fibronectin), and matrigel and fragments were analyzed by SDS-PAGE. Additionally, *in vivo* ECM organization of envenomed rat skin was investigated by immunohistochemistry and hystochemistry. Fibroblast cell migration and wound healing was evaluated by the cell scratch assay. LOBE was able to degrade all tested ECM substrates in a time-dependent way. It was also observed impairment on fibroblast migratory behavior. Analysis of envenomed skin showed an increase on inflammatory cells in the connective tissue surrounding the local of LOBE injection, which was accompanied by changes on collagen organization and degradation of laminin on blood vessel basement membrane. In conclusion, *Lonomia obliqua* bristle extract has a proteolytic activity on extracellular matrix proteins, which might play a role on local and systemic hemorrhage and the appearance of ecchymosis.

Key Words: metaloprotease, caterpillar venom, laminin, collagen, fibronectin

1 Introduction

Venomous animals is the term that denominates animals capable of producing poison or poisonous secretions. There are several classes including thousands of species like snakes, spiders, frogs, caterpillars, scorpions and others. These animals are worldwide distributed and are cause of accidents with humans in tropical and subtropical regions, where the abundance of species is greater. World Health Organization has included poisoning by snake bite as a recognized neglected tropical disease, for the first time in 2009, showing the importance of studies in this area. (Williams,et al., 2010).

Animal poisons are unique and complexes mixtures of proteins and bioactive peptides. These physic-chemicals and biological properties resulted from evolutionary process of species providing such individuals with effective means of protection for defense and feeding on prey and predators. The animal toxins are able to interact with enzymes, receptors and ion channels causing destabilization of physiological systems that are essentials to the victims and prey survival. (Calvete, 2009).

The larvae or caterpillars are larval forms from butterflies and moths (Order Lepidoptera) and accidents involving caterpillars occur in all Brazilian territory. However, the South region is the one which presents the higher incidence of accidents, 7.3/100.000 inhabitants. The most important occurrences are with caterpillars that belong to the genus *Lonomia*. From 4,028 accidents caused by these animals occurred in 2009, 14.5% were caused by *Lonomia* (585/4.028) (SVS, 2010). The specie *Lonomia Obliqua* is the most relevant specie in the South of Brazil (Gamborgi, et al. 2012).

Caterpillar, unlike other venous animals, does not have a specialized gland for poison production, being this used only for defense, not for hunting and feeding. These animals have a secretory epithelium responsible for production of a poisonous secretion which is injected in the victims when the contact breaks the spikes. (Veigas, et al. 2001)

Between two and seventy two hours after a contact the symptoms appear. Bruising and bleeding being characterized as epistaxis, otorrhagia, rectal bleeding, menorrhagia and widespread bruising are the most common symptoms. Besides, in severe cases, the progress of the disease can lead to acute renal failure and intracerebral bleeding. (Duarte,et al. 1990, Kellen, et al. 1995; Duarte, et al.1996). Pro-clotting manifestations can occur in addition to the bleeding symptoms. (Reis, et al. 1999; Zannin, et al. 2003).

Rats envenomed with *L. obliqua* venom show important biochemical, hematological and histopathological alterations, suggesting the occurrence of multi-organ damage and confirming that the rat is a good animal model for studying hemorrhagic disturbances, as well as organ specific injuries. (Berger,et. al. 2013).

Snake venom metalloproteinases hydrolyze key peptide bonds in basement membrane (BM) and supporting extracellular matrix (ECM) components, promoting the weakening of the mechanical stability of BM. As a consequence, the hemodynamic biophysical forces normally operating in the vasculature, such as microvessel wall tension and shear stress, provoke the distention of the weakened capillary, which leads to microvessel disruption and extravasation (Gutierrez, et al., 2005)

The extracellular matrix degradation or remodeling activities exerted by these toxins affect cell–cell and cell–extracellular matrix adhesion and survival and impair inflammatory cell migration into inflamed tissues. (Komegae, et al. 2011). These effects of peptidases on extracellular matrix (ECM) proteins interfere with the normal processes of tissue regeneration and angiogenesis, contributing to tissue damage observed in envenomations. Moreover, the degradation of ECM can induce cells to enter into the apoptotic pathway known as anoikis as consequence of loss of signal transduction between matrix and cells due to a physical disruption of integrin–matrix interactions (Frisch and Screaton, 2001; Grossmann, 2002).

In this work we evaluated the ability of *L. obliqua* bristle extract in degrade extracellular matrix (ECM) components, favoring hemorrhage and impairing wound healing.

2 Material and Methods

2.1 *Lonomia obliqua* venomous secretions

L. obliqua caterpillars were kindly provided by Centro de Informações Toxicológicas (CIT), Porto Alegre, Rio Grande do Sul, Brazil. *L. obliqua* bristle extract was obtained by cutting bristles at the caterpillar's tegument insertion and the excised material was kept at 4°C until preparation of the extract, which occurred immediately after dissection. Bristles were macerated in cold saline solution (150 mM NaCl) and centrifuged at 9600g for 20 min. The protein content of the supernatant, designed as crude *L obliqua* bristle extract (LOBE), was determined by the BCA assay kit (Pierce,Rockford,USA) and aliquots were stored at -80°C until use. For the experiments, four different preparations of bristle extract were used and the total number of caterpillars varied between 50 and 80 specimens per preparation.

2.2 Experimental animals

Male Wistar rats (weighing 300–350 g) were housed in a temperature-controlled room (21–25°C, in a 12-h light/ dark cycle), with free access to water and food. All procedures involving animals were carried out in accordance with the Guiding Principles in the Use of Animals in Toxicology (International Society of Toxicology, http://www.toxicology. org) and the Brazilian College of Animal Experimentation (COBEA). Experiments also followed the recommendations of Ethical Committee for the

Use of Animals of Federal University of Rio Grande do Sul, Brazil. All efforts were made to minimize the number of animals used and their suffering.

Time course of experimental envenomation In order to follow the evolution of platelet and blood coagulation disturbances we developed an experimental model of envenomation in rats (Berger. et al. 2010). Animals were divided into two groups: an experimental group, which was injected subcutaneously (s.c.) with 1mL of a solution containing 1 µg protein of crude LOBE per kg of body weight, and a control group that received subcutaneously 150 mM NaCl in a volume of 1mL, under the same conditions. Two hours after injection, rats were anesthetized and sacrificed. Skin and muscle from the injection site were collected for histological evaluation. A total of 8 animals were utilized in each group.

2.3 Histopathological analyses

All animals from the control and envenomed groups (at each sampling time) were necropsied and gross macroscopic alterations were examined. The skin, muscle and endothelium (at the site of venom injection) were then carefully removed and fixed in a 10% neutral buffered formaldehyde solution. The tissues were dehydrated in gradual alcohol, cleared in xylene and embedded in paraplast. Subsequently, the samples were sectioned and stained with hematoxylin and eosin (H&E), Masson's trichrome and Picrossirius for further analysis by light microscopy.

2.4 Immunohistochemistry

Paraplast embedded samples were cut (3µm), mounted on slides, deparaffinized in xylene and hydrated in ethanol solutions and 0.1M phosphate-saline buffer (PBS), pH 7.4, room temperature (RT). The endogenous peroxidase activity was inactivated by incubation with 0.3% H_2O_2 in Methanol (10min, RT). For antigen recovery, slides were

heated in 0.01 M citrate buffer in a water bath at 95ºC (pH 6.0) for 15 min. The sections were incubated with primary antibodies anti-laminin (Sigma, polyclonal antibody, 1:100, 4ºC, overnight), washed with PBS, incubated with HRP-dual link (Dako) and visualized with DAB chromogen (3',3-diaminobenzidine,Dako).

2.5 Extracellular matrix (ECM) protein degradation

Fibronectin (Sigma), laminin (Sigma), matrigel (Sigma) was incubated with LOBE (10:1 ratio) in Tris-HCl buffer (20 mM, pH 7.4, 37ºC). After specified time points (15min, 60min,120min, 240min and 24h), samples were boiled (95ºC, 5 min), precipited by acetone precipitation, submitted to a 10% SDS-PAGE gel and stained for Comassie blue for analysis of ECM degradation.

2.6 Wound healing assay

NIH 3T3 cells were cultured near confluence, starved overnight and a scratch was performed with a plastic tip. Media were replaced by complete media supplemented with LOBE and the wound clousure was measured after 4 or 8h.

2.7 Angiogenesis assay

It was performed *in vivo* by the ECM gel/sponge assay. Foam sponge soaked in ECM gel in the presence or absence of LOBE was implanted intradermally in rats. After 12 days, the sponge was surgically recovered, macerated in water and blood vessels content was measured by hemoglobin quantification at 540nm.

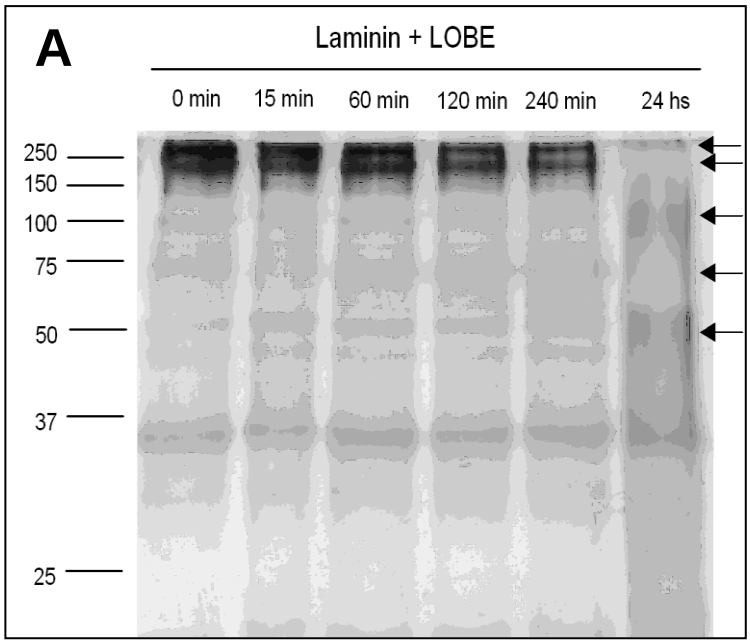
2.8 Statistical analysis

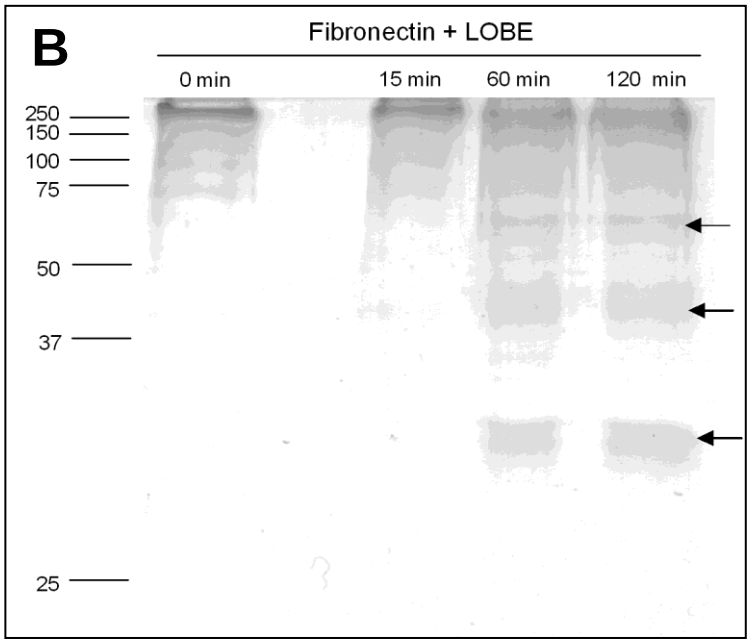
Values are expressed as mean an standard deviation e data was submitted to Student t-test.

3. Results

3.1 LOBE induces in vitro degradation of ECM proteins

Laminin incubated without LOBE showed two main bands of 250 and 200kDa. Partial degradation was observed after 24h incubation with LOBE by the presence of a low-molecular-weight fragment of 100, 75 and 50 kDa. (Fig. 1A). The LOBE also hydrolyzed fibronectin as evidenced by the appearance of new protein bands. After 60 and 120 minutes of incubation the high-molecular band was completely digest by LOBE resulting in several degradation products (Fig. 1B). Matrigel or ECM gel (gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) murine sarcoma cells) also was verified a decreases in concentration after reaction with ECM, after 60, 120 and 360min. (Fig.1C).





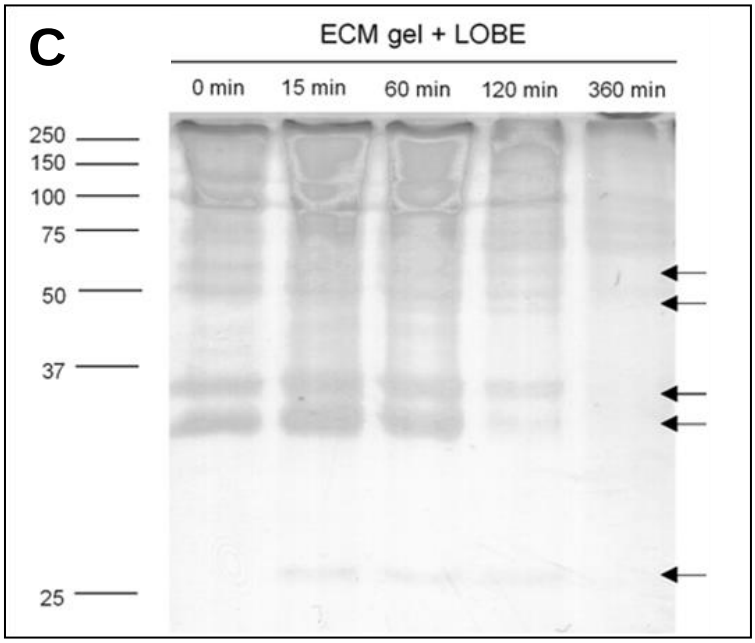


Figure 1 Proteolytic degradation of laminin (A), fibronectin (B) and extracellular matrix derived matrix (C). Samples were incubated with LOBE (10:1 ratio) and analyzed by SDS–PAGE 10%. Arrow indicates the degradation product of reaction.

3.2 LOBE decreases cell migration and angiogenesis

Impairment of cell migration and wound healing was evaluated in vitro by the cell scratch assay. The LOBE induced an impairment on the cell migration process (Fig. 2). The *in vivo* analysis for angiogenesis demonstrated that LOBE induced a decrease on blood vessel formation when compared to the control group (Fig.3).

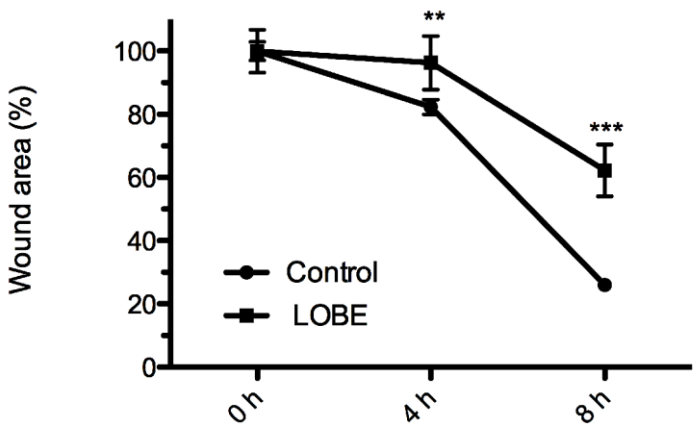


Figure 2- The LOBE decreases cells migration after 4h and 8h compared to the control group. NIH 3T3 fibroblasts were submitted to wound healing assay in the presence or absence of LOBE (1 μg/ml). Student T-test (** $p<0.05$, *** $p<0.01$ n=3)

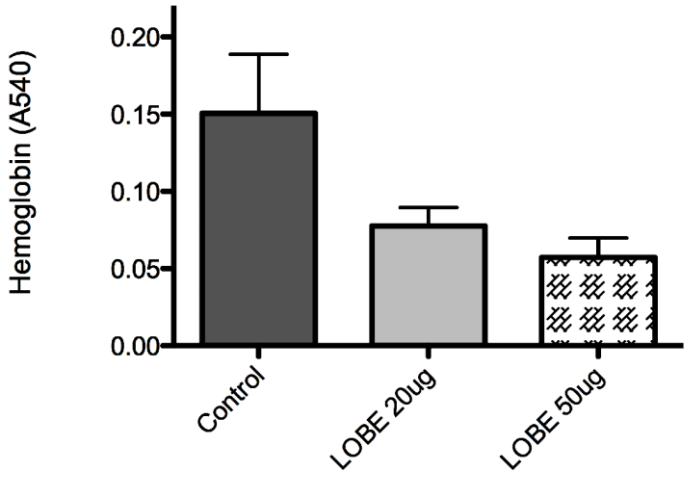


Figure 3: *Lonomia Obliqua* venom decreases angiogenesis *in vivo.* Control animals received saline. Animals were sacrificed 12 days after implanted intradermally with LOBE 20µg and 50µg. Angiogenesis assay dermination in ECM gel/ sponge hemoglobin in the samples is quantified calorimetrically at 540 nm in spectrophotometer.

3.3 LOBE induces ECM remodeling in vivo

Skin morphological analysis showed that after 2h of LOBE administration there was the presence of inflammatory cells in the connective tissue surrounding envenomed region as well the presence of blood clot inside capilary and venule vessels (Fig 4B) when compared to skin exposed to PBS (Fig 4A). Masson Trichrome (Fig 4C and 4D) and Picrossirius (Fig 4E and 4F) staining showed dissociation of collagen fibers in the dermis due to marked edema after LOBE envenomation (Fig. 4D) and changes in the collagen organization and degradation (Fig. 4F). In control rats (Fig 4G), it was observed laminin staining on the basement membrane of blood vessel and, 2h after LOBE administration, there was a more punctuate distribution of this protein, specially on venous vessels (Fig 4H).

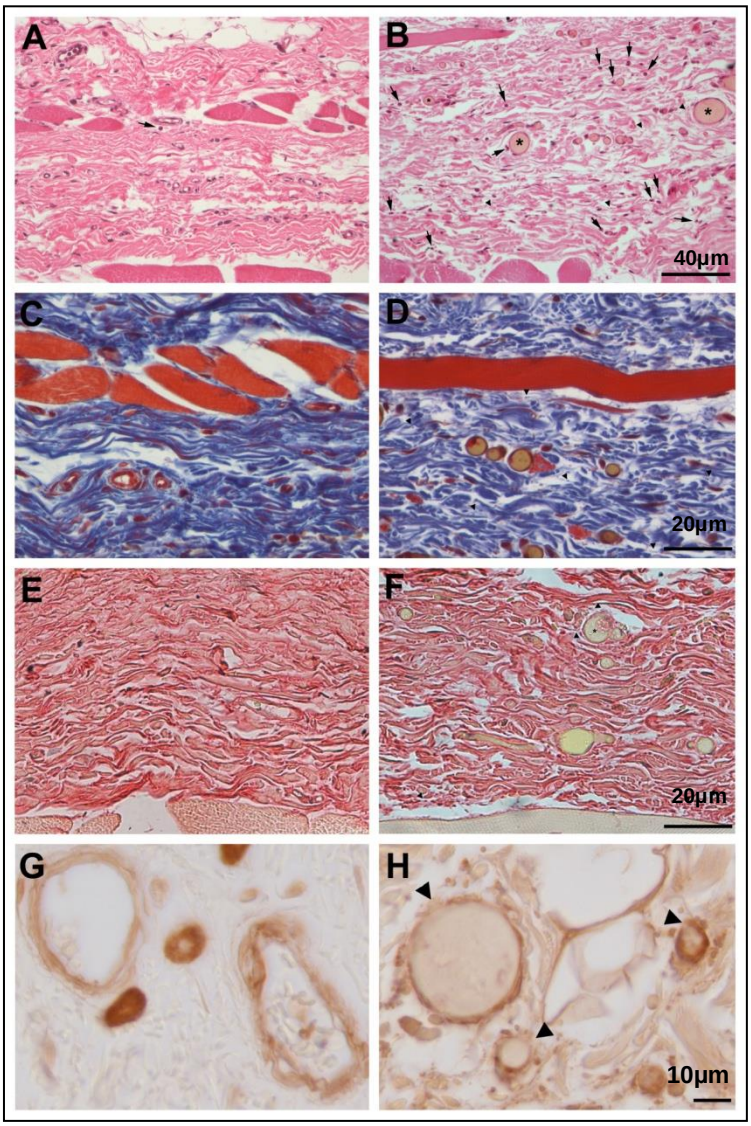


Fig.4 Histologic alterations after L. obliqua envenomation in rats. Representative images of: **A, C, E, F** control animal (one that had been injected with PBS solution, s.c.), showing normal morphology. **B, D, F, H** the animal that had been injected with LOBE (1 mg/kg, s.c.) after 2 h of envenomation. **B.** Hematoxilin/eosin (HE). **D.** Masson Trichrome and **F.** Picrossirius **H.** IHC of Laminin.

4. Discussion

The *L. obliqua* venom, at low and non-hemorragic doses, exerts a direct pro-inflammatory effect on endothelial cells, promoting cytoskeleton reorganization, increasing focal adhesion and the expression of crucial molecules to the onset of a vascular inflammatory response. (Nascimento-Silva, et al. 2012). The poisons have been studied with the objective of better understand its structure and functionality, as well as their protein toxins individually. Consequently, understanding the mechanism and origin of the toxic effect. This is accomplished by isolation of proteins, followed by its biochemical characterization, and then determining the biological activity *in vitro* and *in vivo.* (Gallagher, et al. 2003) The venom are comprises several families of proteins, especially metalloproteases, serine proteases, phospholipases, disintegrins, lectins, and L-amino oxidase .For each family there are a number of isoforms of these proteins that act synergistically with high specificity, composing, on the whole, the biochemical basis of the processes leading to the development of the pathological. (Terra, et al. 2009).

In this study, we demonstrated that LOBE administration is able to alter cell migration and the process of angiogenesis. The venom component of bristle extract of *L. Obliqua*: prothrombin activator (Lopap) acts on the cell preservation, since it regulates the expression of molecules, such as nitric oxide (NO) and prevents cell death. (Fritzen, et al. 2005; Reis, et al. 1999). As well, the component Losac (a factor X activato) that besides its pro-coagulant activity, also functions as a growth factor and an inhibitor of cell death of endothelial cells. Possibly, TPA (tissue plasminogen activator) and NO stimulated by Losac, induces cell preservation, since the release of TPA and NO can inhibit the proliferation and induce apoptosis of endothelial cells. So that both are related to angiogenesis. (Alvarez-Flores, et al. 2006).

New blood vessel growth, or angiogenesis, occurs during the development and maturation as well as during critical physiological processes, including wound healing and reproduction. However, angiogenic processes are also usurped in many pathologies, and thereby contribute to an array of disorders including cancer, inflammation and autoimmune diseases (Folkman, 2006). Following regression of native vessels, degradation of the vessel basement membrane and surrounding ECM to allow for invasion of endothelial cells is an integral part of the ongoing angiogenic process. After regression of existing vessels and breakdown of the basement membrane, endothelial cells proliferate and begin migrating. In toward tumor cells expressing pro-angiogenic compounds. (Carmeliet & Jain, 2000, 2011). The LOBE decreases the formation of new vessels and also cause rupture of the existent.

Extracellular matrices are composed of dynamic and highly ordered structural networks of collagens, proteoglycans and noncollagenous glycoproteins. The temporal and spatial regulation of ECM formation contributes to the mechanical and biological properties of different connective tissues and has fundamental effects on cellular phenotypes (Nelson and Bissell, 2006). ECM components also regulate the local release of growth factors and play an important role on cell differentiation, migration and adhesion by providing linkages between cells and the ECM (Heinegard,et al. 2002). In this work, we demonstrated *in vitro* and *in vivo* that LOBE has a proteolitic acitivy upon collagen, laminin and fibronectin substrates. It was already reported that metalloproteinases and serine protease-like presented in *L. achelous* hemolymph also can degrade extracellular matrix proteins such as laminin, vitronectin and fibronectin. These toxins were identified as chromatographic fractions FDII, Lonomin V and Lonomin V-2. These toxins may contribute to the hemorrhagic events triggered by *L. achelous* venom because they may facilitate the spreading of the venom through the victim's body and aggravate hemorrhage by destroying the capillaries. (Lucena, et al.

2006). *In vitro* assays that use high protease to substrate ratios have shown that MMPs can cleave a wide range of ECM molecules. This has led to the widely-held concept that MMP mediated cleavage of ECM molecules is the means by which infiltrating cells, including leukocytes and transformed cells, penetrate ECM barriers and that MMPs are essential for all remodelling processes in developing or regenerating tissues. (Sorokin, 2010).

Basement membrane constitutes a complex extracellular matrix scaffold, composed mainly of collagen IV, laminin, nidogen and the proteoglycan perlecan, that provides support to endothelial cells in blood vessels and plays various roles in the communication between intracellular and extracellular environments (Martin and Timpl, 1987). Interactions between laminin , Fibronectin and collagen, and other components of basement membranes are crucial for both biological and mechanical properties of the skin (von der Mark, et al. 1992). In this work, we demonstrated evidence of laminin degradation on basement membrane of small blood vessels of envenomed skin. This data suggest that the proteolytic activity of LOBE might play an important role during the hemorrhage process. It is also possible that during the ECM remodeling induced by LOBE there is a release of small peptides that might work as signaling and chemoattractive molecules for leukocytes, as observed by HE staining. In fact, it was already showed that several molecules resulting from laminin cleavage have this property. (Sorokin, 2010, Nascimento-Silva, et al. 2012). The fibronectin analysis confirmed the degradatory activity of venom upon this glycoprotein, even when it was organized on the extracellular matrix produced by endothelial cells. (Paludo, et al., 2006)

5. Conclusion

Accidents with *Lonomia obliqua* are mostly characterized by bleeding as consequence of a severe hemorrhagic syndrome caused by a complex coagulopathic process that can easily evolve to death if not treated in the very beginning of the accident occurrence. Since the caterpillar venom is not synthesized by a gland, the toxic compounds are frequently derived from a mixture of a secretion produced by the specialized epithelium.

The LOBE caused morphological alterations. It was demonstrated that the LOBE caused Laminin, fibronectin and collagen degradation, observed *In vitro* and *In vivo*. It happened diapedesis and the rupture of basal membrane of small blood vessels. Beyond, a decrease on fibroblast migration and angiogenesis. Degradation of ECM by *L. obliqua* proteases may be a key factor in facilitating systemic hemorrhage and the appearance of ecchymosis.

Acknowledgments

This work was supported by funding and fellowships from the Brazilian Agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Ministério da Educação, Brazil (CAPES-MEC) – Edital Toxinologia (Processo: 23038.006277/2011-85).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Alvarez-Flores, M.P., Fritzen, M., Reis, C.V., Chudzinski-Tavassi, A.M. 2006. Losac, a factor X activator from Lonomia oblique bristle extract: its role in the pathophysiological mechanisms and cell survival. Biochemical and Biophysical Research Communications 343, 1216-1223.

Berger, M, Reck Jr., J, Terra, R.M.S., Pinto, A.F.M., Termignoni, C., Guimarães, J., A. 2010. Lonomia obliqua caterpillar envenomation causes platelet hypoaggregation and blood incoagulability in rats. Toxicon 55, 33-44,

Berger, M., Beys-da-Silva, W. O., Santi, L., Oliveira, I. M., Jorge P.M., Henriques, J.A.P., Driemeier, D., Vieirae, M.A.R., Guimarães,J.A. 2013 Acute Lonomia oblique caterpillar envenomation-induced physiopathological alterations in rats: Evidence of new toxic venom activities and the efficacy of serum therapy to counteract systemic tissue damage. Toxicon 74, 179–192.

Calvete, J. J. 2009. Venomics: digging into the evolution of venomous systems and learning to twist nature to fight pathology. Journal Proteomics, 72, 121-6.

Carmeliet P, Jain, R.K. 2000. Angiogenesis in Cancer and Other Diseases. Nature 407, 249-257.

Carmeliet P, Jain, R.K. 2011. Molecular mechanisms and clinical applications of angiogenesis. Nature 473, 298-307.

Duarte, A.C, Crusius, P.S., Pires, C.A., Schilling, M.A., Fan, H.W. 1996. Intracerebral haemorrhage after contact with *Lonomia* caterpillars. Lancet 348, 1033.

Duarte, A.C., Caovilla, J., Lorini, I., Lorini, D., Mantovani, G.,Sumida, J., Manfre, P.C., Silveira, R.C., Dr Moura, S.P. 1990. Insuficiência renal aguda por acidentes com lagartas. Jornal Brasileiro de Nefrologia 12, 184-186.

Folkman, J. 2006. Angiogenesis .Annul Reviews Medicine 57: 1–18.

Frisch, S.M. Screaton R.A. 2001. Anoikis mechanisms. Curr. Opin. Cell Biol., 13, 555–562.

Fritzen, M., Flores, M.P.A., Reis, C.V., Chudzinski-Tavassi, A.M. 2005. A prothrombin activator (Lopap) modulating inflammation, coagula-tion and cell survival mechanism, Biochemical and Biophysical Research Communications 333, 517-523.

Gallagher, P.G.;Bao, Y.; Serrano, S M.T; Kamiguti A.S.; Theakston, R. D. G.; Fox, J.W. 2003. Use of microarrays for investigating the subtoxic effects of snake venoms: insights into venom-induced apoptosis in human umbilical vein endothelial cells. Toxicon 41, 429–440.

Gamborgi, G.P., Coelho, A.M., Rossetto, D. S., Busato, M.A. 2012. Influência dos fatores abióticos sobre casos de acidentes provocados por *lonomia obliqua*. Hygeia 14, 201-208.

Grossmann, J. 2002. Molecular mechanisms of “detachment-induced apoptosis-Anoikis”. Apoptosis 7, 247–260.

Gutierrez, J.M., Rucavado, A., Escalante, T., Diaz, C. 2005. Hemorrhage induced by snake venom metalloproteinases: biochemical and biophysical mechanisms involved in microvessel damage. Toxicon 45: 997–101

Heinegard, D., Aspberg, A., Franzen, A., Lorenzo, P. 2002. Glycosylated matrix proteins. In: Royce, P.M., Steinman, B. (Eds.), Connective Tissue and its Heritable Disorders. Wiley Liss, New York, 271–291.

Kelen, E.M.A., Picarelli, Z.P., Duarte, A.C. 1995. Hemorrhagic syndrome induced by contact with caterpillars of the genus *Lonomia* (Saturniidae, Hemileucinae). Jornal of Toxicology 14, 283-308.

Komegae, E.N., Ramos, A. D., Oliveira, A.K., Serrano, S.M.T., Lopes-Ferreira,M., Lima, C. 2011. Insights into the local pathogenesis induced by fish toxins: Role of natterins and nattectin in the disruption of cell–cell and cell–extracellular matrix interactions and modulation of cell migration. Toxicon, Volume 58, 509-517.

Lucena, B., Guerrero, A.M., Salazar, A.. Gil, C.L. Arocha-Piñango, A. 2006. Degradation of extracellular matrix proteins (fibronectin, vitronectin and laminin) by serine-proteinases isolated from Lonomia achelous caterpillar hemolymph Blood Coagul. Fibrinolysis, 17, 427–435.

Martin G.R., Timpl R. 1987. Laminin and other basement membrane components, Annul. Rev. Cell Biol. 3, 57–85.

Nascimento-Silva, V.,, Da Silva, G.R., Moraes, J.A., Cyrino, F.Z.; Seabra, S.H.; Bouskela, E., Guimarães, J.A., Barja-Fidalgo, C. 2012 . A pro-inflammatory profile of endothelial cell in Lonomia obliqua envenomation. . Toxicon 60, 50-60

Nelson, C.M., Bissell,M.J. 2006. Of extracellularmatrix, scaffolds, and signaling: tissue architecture regulates development, homeostasis, and cancer. Annu. Rev. Cell Dev. Biol. 22, 287–309.

Paludo, K.S., Gremskia, L.H., Veiga, S.S,. Chaimd, O.M, Gremskic,w, Buchi, D.F., Nader,H.D., Dietrich, C.P., Franco, C.R.C. 2006. The effect of brown spider venom on endothelial cell morphology and adhesive structures. Toxicon 47, 844–853.

Reis, C.V., Kelen, E.M.A., Farsky, S.H.P., Portaro, F.C.V., Sampaio, C.A.M., Fernandes, B.L., Camargo, A.C.M., Chud-zinski-Tavassi, A.M. 1999. A Ca^{++} activated serine protease (LOPAP) could be responsible for the haemorrhagic syndrome caused by the caterpillarLonomia obliqua. The Lancet 353, 1942.

Secretaria de Vigilância em Saúde, Ministério da Saúde (SVS). 2010. Situação Epidemiológica das Zoonoses de Interesse à Saúde Pública. Boletim Eletrônico Epidemiológico 9. pages: 21-22. Brasília.

Sorokin, L. 2010. The impact of the extracellular matrix on inflammation. Nature reviews - immunology 10,712-723.

Terra, R. M., Pinto, A. F., Guimaraes, J. A. , Fox, J. W. 2009. Proteomic profiling of snake venom metalloproteinases (SVMPs): insights into venom induced pathology. *Toxicon,* 54**,** 836-44.

Veiga, A.B.G., Blochtein, B., Guimarães, J.A. 2001. Structures involved in production, secretion and injection of the venom produced by the caterpillar Lonomia obliqua (Lepidoptera, Saturniidae). Toxicon 39, 1343-1351.

Von Der Mark, K., Von Der Mark, H., Goodman, S.1992 Cellular responses to extracellular matrix Max-Planck-Society, Clinical Research Units for Rheumatology at the Clinic III and Nephrology Unit at the Clinic IV of the University of Erlangen, Erlangen, Germany Kidney International 41,632-640.

Williams, D., Gutierrez, J. M., Harrison, R., Warrell, D. A., White, J., Winkel, K. D. & opalakrishnakone, P. 2010. The Global Snake Bite Initiative: an antidote for snake bite. The Lancet 375**,** 89-91

Zannin, M., Lourenc¸o, D.M., Motta, G., Costa, L.R.D., Grando, M., Gamborgi, G.P., Noguti, M.A., Chudzinski-Tavassi, A.M. 2003. Blood coagulation and fibrinolytic factors in 105 patients with hemorrhagic syndrome caused by accidental contact with Lonomia oblique caterpillar in Santa Catarina, Southern Brazil. Thrombosis and Haemostatis 89, 355–364.

Anexo 1

TOXICON

An Interdisciplinary Journal on the Toxins Derived from Animals, Plants and Microorganisms

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0041-0101

DESCRIPTION

Toxicon's "aims and scope" are laid down in the journal as:

To publish:
articles containing the results of original research on problems related to toxins derived from animals, plants and microorganisms papers on novel findings related to the **chemical**, **pharmacological**, **toxicological**, and **immunological** properties of natural **toxins molecular biological** studies of toxin and other **genes** from **poisonous** and **venomous** organisms that advance understanding of the role or function of toxins clinical observations on poisoning and envenoming where a new therapeutic principle has been proposed or a decidedly superior clinical result has been obtained material on the use of toxins as tools in studying biological processes and material on subjects related to **venom** and **antivenom** problems review articles on problems related to **toxinology.**

And
To encourage the exchange of ideas, sections of the journal may be devoted to Short Communications, Letters to the Editor and activities of the International Society on Toxinology.

Toxicon strives to publish articles that are current and of broad interest and importance to the toxinology research community. Emphasis will be placed upon articles that further the understanding and knowledge of toxinology.

Types of paper

Full-Length Research Papers: Articles containing the results of original research on problems related to toxins derived from animals, plants and microorganisms.

Short Communications: Short communications differ from full manuscripts only in that the research study does not lend itself to an extended presentation. Even though brief, the Short communication should represent a complete, coherent and self contained study. The quality of Short Communications is expected to be as good as that of full articles, and both full articles and Short communications will be refereed in an identical manner. The form is identical to that for a full article except that the report should not be divided into Introduction, Materials and Methods, Results and Discussion. An abstract of not more than 75 words should be provided. The Short Communication may not be longer than five double-spaced typewritten pages (not including references, tables and figures) and should include not more than two tables of two figures or one of each.

Letters to the Editor: These may be published if judged by the Editor to be of interest to the broad field of toxinology or of special significance to a smaller group of workers in a specialized field of toxinology. They should be headed `Letter to the Editor' which should be followed by a title for the communication. Names of authors and affiliations should be at the end of the letter.

Announcements: *Toxicon* will only accept for publication announcements of great interest to toxinologists, such as notices of appropriate meetings and symposia and activities of the International Society of Toxinology.

Reviews and mini-Reviews: Articles of interest to toxinologists which are published in journals other than *Toxicon* may be abstracted in the Reviews section of *Toxicon*. Readers who feel that a particular article or book should be abstracted in this section are encouraged to bring their opinions to the attention of one of the Review Editors. Mini-Reviews and proposals for mini-Reviews are welcome

Molecular Biology: Papers on molecular biological aspects of toxins are welcome. They can include cloning, expression, genetic and related studies. The papers must add to the understanding of the role or function of toxins. Papers providing cDNA sequences without any relevant conclusions are not acceptable. If cDNA sequences are included, authors must guarantee that the sequences will be deposited in a public gene bank before the publication of the paper in *Toxicon*.

Clinical reports: *Toxicon* will publish clinical reports on poisoning where a new therapeutic principle has been proposed or a decidedly superior clinical result has been established. Please observe the following: Clinical Reports Guidelines

Classic Toxins: The main aim of these articles is to educate and inform both the experienced scientist and new scientists entering the field of toxinology. These articles should serve as a reference guide for anyone using toxins. Please contact Dr. Ed Rowan with your suggestions for inclusion in the 'classic toxins' feature.

AUDIENCE

Toxicologists, toxinologists, molecular biologists and chemists.

IMPACT FACTOR

2012: 2.924 © Thomson Reuters Journal Citation Reports 2013

ABSTRACTING AND INDEXING

BIOSIS
Cambridge Scientific Abstracts
Chemical Abstracts
Current Contents/BIOMED Database
Current Contents/Life Sciences
Current Contents/SciSearch Database
Current Contents/Science Citation Index
EMBASE
EMBiology
Elsevier BIOBASE
MEDLINE®
PASCAL/CNRS
Research Alert
Scopus

EDITORIAL BOARD

GUIDE FOR AUTHORS

Your Paper Your Way
ypyw-gfa-banner.gifyour paper your way

INTRODUCTION

Official Journal of The International Society on Toxinology (http://www.toxinology.org/), *Toxicon*'s "aims and scope" are laid down in the journal as:

To publish:
articles containing the results of original research on problems related to toxins derived from animals, plants and microorganisms papers on novel findings related to the chemical, pharmacological, toxicological, and immunological properties of natural toxins molecular biological studies of toxin and other genes from poisonous and venomous organisms that advance understanding of the role or function of toxins clinical observations on poisoning and envenoming where a new therapeutic principle has been proposed or a decidedly superior clinical result has been obtained material on the use of toxins as tools in studying biological processes and material on subjects related to venom-antivenom problems review articles on problems related to toxinology.

And
To encourage the exchange of ideas, sections of the journal may be devoted to Short Communications, Letters to the Editor and activities of the International Society on Toxinology.

Toxicon strives to publish articles that are current and of broad interest and importance to the toxinology research community. Emphasis will be placed upon articles that further the understanding and knowledge of toxinology.

Types of paper

Full-Length Research Papers: Articles containing the results of original research on problems related to toxins derived from animals, plants and microorganisms.

Short Communications: Short communications differ from full manuscripts only in that the research study does not lend itself to an extended presentation. Even though brief, the Short communication should represent a complete, coherent and self contained study. The quality of Short Communications is expected to be as good as that of full articles, and both full articles and Short communications will be refereed in an identical manner. The form is identical to that for a full article except that the report should not be divided into Introduction, Materials and Methods, Results and Discussion. An abstract of not more than 75 words should be provided. The Short Communication may not be longer than five double-spaced typewritten pages (not including references, tables and figures) and should include not more than two tables of two figures or one of each.

Letters to the Editor: These may be published if judged by the Editor to be of interest to the broad field of toxinology or of special significance to a smaller group of workers in a specialized field of toxinology. They should be headed `Letter to the Editor' which should be followed by a title for the communication. Names of authors and affiliations should be at the end of the letter.

Announcements: *Toxicon* will only accept for publication announcements of great interest to toxinologists, such as notices of appropriate meetings and symposia and activities of the International Society of Toxinology.

Reviews and mini-Reviews: Articles of interest to toxinologists which are published in journals other than *Toxicon* may be abstracted in the Reviews section of *Toxicon*. Readers who feel that a particular article or book should be abstracted in this section are encouraged to bring their opinions to the attention of one of the Review Editors. Mini-Reviews and proposals for mini-Reviews are welcome

Molecular Biology: Papers on molecular biological aspects of toxins are welcome. They can include cloning, expression, genetic and related studies. The papers must add to the understanding of the role or function of toxins. Papers providing cDNA sequences without any relevant conclusions are not acceptable. If cDNA sequences are included, authors must guarantee that the sequences will be deposited in a public gene bank before the publication of the paper in *Toxicon*.

Clinical reports: *Toxicon* will publish clinical reports on poisoning where a new therapeutic principle has been proposed or a decidedly superior clinical result has been established. Please observe the following: Clinical Reports Guidelines

Classic Toxins: The main aim of these articles is to educate and inform both the experienced scientist and new scientists entering the field of toxinology. These articles should serve as a reference guide for anyone using toxins. Please contact Dr. Ed Rowan with your suggestions for inclusion in the 'classic toxins' feature.

BEFORE YOU BEGIN

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Conflict of Interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest.

Toxicon requires full disclosure of all potential conflicts of interest. All sources of funding supporting the work are to be declared. At the end of the manuscript text (and in the cover letter of the manuscript), under a subheading "Conflict of Interest statement", all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. If there are no conflicts of interest, the authors should state, "The authors declare that there are no conflicts of interest." Signed copies of the *Toxicon* Conflict of Interest policy form are required upon submission. The Conflict of Interest policy form can be downloaded here. In order to minimize delays, we strongly advise that the signed copies of these statements are prepared before you submit your manuscript. The corresponding author is responsible for sharing this document with all co-authors. Each and every co-author must sign an individual disclosure form. The corresponding author is responsible for uploading their form and those of their co-authors.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:
Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.
After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

This journal offers authors a choice in publishing their research: Open Access and Subscription.

For Subscription articles
Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.
Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For Open Access articles
Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Retained author rights
As an author you (or your employer or institution) retain certain rights. For more information on author rights for:
Subscription articles please see http://www.elsevier.com/journal-authors/author-rights-and-responsibilities.
Open access articles please see http://www.elsevier.com/OAauthoragreement.

Role of the funding source
You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see http://www.elsevier.com/funding.

Funding Body Agreements and Policies
Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

US National Institutes of Health (NIH) voluntary posting (" Public Access") policy:
Elsevier facilitates author response to the NIH voluntary posting request (referred to as the NIH "Public Access Policy"; see http://www.nih.gov/about/publicaccess/index.htm) by posting the peer-reviewed author's manuscript directly to PubMed Central on request from the author, 12 months after formal publication. Upon notification from Elsevier of acceptance, we will ask you to confirm via e-mail (by e-mailing us at NIHauthorrequest@elsevier.com) that your work has received NIH funding and that you intend to respond to the NIH policy request, along with your NIH award number to facilitate processing. Upon such confirmation, Elsevier will submit to PubMed Central on your behalf a version of your manuscript that will include peer-review comments, for posting 12 months after formal publication. This will ensure that you will have responded fully to the NIH request policy. There will be no need for you to post your manuscript directly with PubMed Central, and any such posting is prohibited.

Open access
This journal offers authors a choice in publishing their research:

Open Access
• Articles are freely available to both subscribers and the wider public with permitted reuse
• An Open Access publication fee is payable by authors or their research funder
Subscription
• Articles are made available to subscribers as well as developing countries and patient groups through our access programs (http://www.elsevier.com/access)

• No Open Access publication fee

All articles published Open Access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by your choice of one of the following Creative Commons user licenses:
Creative Commons Attribution (CC BY): lets others distribute and copy the article, to create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.
Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA): for non-commercial purposes, lets others distribute and copy the article, to create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, do not modify the article in such a way as to damage the author's honor or reputation, and license their new adaptations or creations under identical terms (CC BY-NC-SA).
Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND): for non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

To provide Open Access, this journal has a publication fee which needs to be met by the authors or their research funders for each article published Open Access.
Your publication choice will have no effect on the peer review process or acceptance of submitted articles.

The publication fee for this journal is **$3,000**, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission
Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Referees
The Editors welcome submissions by the authors of the names and addresses of up to five individuals who could expertly review the paper, and who are not from the same institutions as the authors. The Editors reserve the right to use these or other reviewers.

PREPARATION

NEW SUBMISSIONS
Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.
As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality

figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.
If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.
Divide the article into clearly defined sections.

Please ensure the text of your paper is double-spaced and has consecutive line numbering– this is an essential peer review requirement.

Figures and tables embedded in text
Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

REVISED SUBMISSIONS

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Experimental
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 125 characters including spaces, or, maximum 20 words per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Database linking
Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Math formulae
Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.
Table footnotes
Indicate each footnote in a table with a superscript lowercase letter.

Artwork
Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.
A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.** Please indicate your preference for color: in print or on the Web only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications which can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be

applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source
Journal names should be abbreviated according to the
List of title word abbreviations: http://www.issn.org/2-22661-LTWA-online.php.

Video data
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides
The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary data
Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should

submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address
• Telephone

All necessary files have been uploaded, and contain:
• Keywords
• All figure captions
• All tables (including title, description, footnotes)

Further considerations
• Manuscript has been 'spell-checked' and 'grammar-checked'
• All references mentioned in the Reference list are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Web)
• Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
• If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):

http://dx.doi.org/10.1016/j.physletb.2010.09.059

When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Online proof correction

Corresponding authors will receive an e-mail with a link to our ProofCentral system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately - please upload all of your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail (the PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use). For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com/myarticleservices/offprints).

Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/offprints/myarticlesservices/booklets).

AUTHOR INQUIRIES

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage. For detailed instructions on the preparation of electronic artwork, please visit http://www.elsevier.com/artworkinstructions. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at http://www.elsevier.com/trackarticle. You can also check our Author FAQs at http://www.elsevier.com/authorFAQ and/or contact Customer Support via http://support.elsevier.com.