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Leucurogin, a new recombinant disintegrin cloned from *Bothrops leucurus* (white-tailed-jararaca) with potent activity upon platelet aggregation and tumor growth

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ABSTRACT

Disintegrins and disintegrins-like proteins are able to inhibit platelet aggregation and integrin-mediated cell adhesion. The aim of this study was to produce one disintegrin-like cloned from *Bothrops leucurus* venom gland and to characterize it regarding biological activity. The recombinant protein was purified by one step procedure involving anion-exchange chromatography (DEAE-cellulose) and presented a molecular mass of 10.4 kDa. The purified protein was able to inhibit platelet aggregation induced by collagen ($IC_{50} = 0.65$ µM) and to inhibit growth of Ehrlich tumor implanted in mice by more than 50% after 7 days administration of 10 µg/day. No effects were observed upon adenosine 5′-diphosphate (ADP)-and arachidonic acid (AA)-induced platelet aggregation. The recombinant protein was recognized by an antibody specific for jararhagin one metalloproteinase isolated from *Bothrops jararaca* venom, and therefore it was named leucurogin. Anti-angiogenesis effect of leucurogin was evaluated by the sponge implant model. After 7 days administration leucurogin inhibited, in a dose dependent way, the vascularization process in the sponge. Leucurogin represents a new biotechnological tool to understand biological processes where disintegrins-like are involved and may help to characterize integrins that can be involved in development and progression of malignant cells.

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1. Introduction

Bothrops snake venoms are a complex mixture of biological active peptides and proteins (Kini and Evans, 1990; Rosing and Tans, 1992), which can cause local and systemic lesions including pain, edema, hemorrhage, tissue necrosis, and blood coagulation disorders (Barraviera, 1994; Fonseca, 2001; Melo et al., 2005; Markland, 1998). Snake venom metalloproteinases (SVMPs) are members of the super family of zinc-dependent proteinases (Jia and Pérez, 2010; Oliveira et al., 2010; Markland, 1998) and are associated with hemorrhagic and fibrinolytic activities of the venoms (Lou et al., 2005). Based on their cDNA and structural domains SVMPs are classified into four major groups: PI to PIV Bjarnason and Fox (2004). Members of class PI (20–30 kDa) contain only the zinc-dependent proteolytic domain. Class PII members (30–50 kDa)

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contain both proteolytic and disintegrin domains. The disintegrin domain presents RGD (Arg-Gly-Asp) or KGD (Lys-Gly-Asp) sequences characteristic of disintegrins, responsible for binding with integrins. Free disintegrins can be found in the snake venom as 5–10 kDa hydrolysis products of class PII members. Class PIII members (50–65 kDa) are comprised of three domains, the proteolytic, the disintegrin-like and the cysteine rich domains. In contrast to PII disintegrins, free disintegrin-like domains are not found in the snake venom. Instead, hydrolysis of class PIII members leads to the production of fragments containing both the disintegrin-like and the cysteine rich (dis-cys) domains (Selistre de Araujo et al., 2005; Cidade et al., 2006). These dis-cys proteins are larger than RGD disintegrins presenting molecular mass in the range of 27–30 kDa. In addition, the disintegrin-like domains present XECD (X-Asn-Cys-Asp) motif instead of RGD/KGD tripeptide characteristic of disintegrins. Class PIV members (95 kDa) have, in addition to the class PIII domains, a lecithin domain.

The participation of integrins in inflammatory process, vascular diseases and cancer is well known. Therefore the characterization of integrins antagonists is an interesting subject of study and disintegrins appears as putative candidates to be used as effective tools for cancer therapy. On the other hand, the biological activity of the conjugate dis-cys is not yet clear. Alternagin C, a 29 kDa dis-cys from *Bothrops alternatus* is able to promote adhesion, migration and endothelial cell proliferation after binding to $\alpha_2ß_1$ integrin (Selistre de Araujo et al., 2005). The $\alpha_2ß_1$ integrin is a major collagen receptor that plays an essential role in the adhesion of normal and tumor cells to the extracellular matrix (Selistre de Araujo et al., 2005). Jararhagin, the most well characterized class PIII metalloproteinase isolated from *Bothrops jararaca* was described to inhibit, *in vitro*, platelet aggregation induced by type I collagen-$\alpha_2ß_1$ integrin interaction (Moura da Silva et al., 2001; Zigrino et al., 2002). Tanjoni et al. (2010) showed that $\alpha_2ß_1$ integrin may interact with two different sites in the jararhagin, the ECD-motif located at the disintegrin-like domain and with another motif located at the cysteine rich domain. The aim of this study was to produce, using *Pichia pastoris* expression system, the disintegrin-like domain from *Bothrops leucurus* SVMP and to determine the activity of this recombinant protein upon platelet aggregation and tumor growth. The recombinant protein, named leucurogin, presents 10.4 kDa and is produced in very high amounts in our yeast system. Our results show that leucurogin is able to inhibit platelet aggregation induced by collagen and Ehrlich tumor growth. In a sponge implant model leucurogin showed to be able to potently inhibit vascularization process.

2. Materials and methods

2.1. Chemical products

DEAE-cellulose was a product from Pharmacia (Uppsala, Sweden). The hollow-fiber system was from GE Healthcare (Uppsala, Sweden). Collagen and ADP were from Helena Laboratories (Beautmont, TX, USA).

2.2. Venom gland and antibody

One gland from an adult *B. leucurus* was collected and stored at −80 °C until use. Polyclonal anti-jararhagin antiserum was kindly supplied by Dr. Ana Moura from Instituto Butantan, Sao Paulo, Brazil and was produced as described by Harrison et al. (2000).

2.3. Animals

Swiss male mice, 25–30 g body weight were used for biological assay. The experiments reported here were performed according to the guidelines established by the Brazilian College for Animal Experimentation (COBEA) and by local animal Ethics Committee.

2.4. Cloning of disintegrin cDNA

Total RNA was extracted from venom gland using Trizol (Promega, Madison, WI, USA). Primer sequences, established considering the disintegrin domain of jararhagin (Paine et al., 1992), contained Xho I restriction site in the KEX2 cleavage site for the sense (CTCGAGAAAAGAGAGGTGGGAGAATGTGAC) and Xba I restriction site followed by stop codon for anti-sense (AGATCTCTACTTATGGAAGACATCTGC). The RT-PCR product was cloned into pGEM-T easy vector (Promega, Madison, WI, USA) and after sequencing it was subcloned into pPIC9 vector (Invitrogen, Carlsbad, California, USA). The sequencing of cDNA was carried out by the BigDye Terminator Ready Reaction Mix kit from Applied Biosystems and resolved in a 3130XL sequencer (Foster, CA, USA). The pPIC9 containing the disintegrin sequence was linearized using Bgl II, the fragment containing the disintegrin segment was purified and used to transform the MDS 1168 *P. pastoris* strain (Invitrogen, Carlsbad, CA, USA) by electroporation (1500 V, 25 μF, 400Ω). Positive clones were identified by replica-plating of colonies on methanol containing plates.

2.5. Expression and purification

For protein expression the procedure was as previously described by Santos et al. (2010). Positive clones were plated on solid yeast extract peptone dextrose (YPD) medium and incubated for 48 h at 30 °C. The cells were inoculated into 25 mL of buffered minimal glycerol (BMGY) medium, pH 6. At DO_{600} between 2 and 6, the cell suspension was centrifuged and the pellet resuspended into 100 mL of buffered minimal methanol (BMM) medium. The protein expression was induced by addition of methanol to a final concentration of 0.5% in the medium. Samples from the medium were collected at time zero and after each 24 h intervals until 72 h. The expressed protein was purified from the fermentation medium by tangential filtration in a hollow-fiber system using a 5 kDa cutoff membrane. The concentrated protein from the tangential filtration was dialyzed against 20 mM Tris–HCl buffer pH 8.4 and loaded in a DEAE-cellulose (2 × 3 cm) column on an FPLC system (Pharmacia, Uppsala, Sweden). The column was equilibrated and eluted with 20 mM Tris–HCl buffer pH 8.4 at a flow rate of 1 mL/min. Adsorbed proteins were

eluted with a stepwise gradient of NaCl concentration (200, 500 and 1000 mM) in the 20 mM Tris–HCl buffer pH 8.4. Protein concentration was estimated using the Proteoquant reagent (Proteobras, SP, Brazil) as described by Bradford (1976) and the bicinchoninic acid method (Smith, 1985).

2.6. Western-blotting

Western blot was performed with denatured protein separated in a 12% sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to a polyvinylidene fluoride membrane (PVDF; Bioagency, Hamburg, Germany). Blots were blocked at room temperature with 2.5% non-fat dry milk in phosphate buffered saline (PBS) plus 0.1% Tween 20 (PBS-T) before incubation with rabbit anti-jararhagin antiserum (diluted 1:2000). The immunecomplexes were detected by chemiluminescent reaction (ECL^+ kit, Amersham GE Healthcare, Uppsala, Sweden).

2.7. Platelet aggregation

Te recombinant protein was tested for the effect upon platelet aggregation using fresh human platelet rich plasma (PRP) as described by Higuchi et al. (2007). A PACKS-4 platelet aggregation chromogenic kinetic system (Helena Laboratories, Beautmont, TX, USA) was used to platelet aggregation monitoring. Inhibition of adenosine 5′-diphosphate (ADP)-, arachidonic acid (AA)-, and collagen-induced platelet aggregation was conducted at 37 °C by adding the recombinant protein (0.5–3 μM final concentration) 3 min before the addition of the agonist (final concentrations: ADP, 10 μM; AA, 30 μg/mL and collagen, 5 μg/mL).

2.8. Ehrlich tumor

Ten days after intraperitoneal inoculation of cells in mice, the ascitic tumor was removed and the cells separated by centrifugation at 3000×g for 3 min. After washing the cells with saline, the cellular viability was determined using Trypan blue. Samples presenting cellular viability lower than 90% were discarded. Viable cells (2.5×10^6) were inoculated subcutaneously in mice and in the eighth day after inoculation the treatment was initiated and lasted seven days with daily subcutaneous injections (Higuchi et al., 2007). Groups of 20 mice were treated with three different doses of purified recombinant protein (5, 10 or 20 μg per animal per day) or 20 μg of protein from fermentation medium without methanol induction. Samples were administered subcutaneously until the 7th day (7 doses) and at the 8th day the animals were sacrificed and the tumor removed and weighed. Animals from the control group received injections of 100 μL 0.9% saline.

2.9. Angiogenesis in sponge disc implant

Angiogenesis was determined indirectly by the sponge implant model in mice (Santos et al., 2010). Polyurethane sponge discs (Vitafoam Ltd., London, UK), 8 mm diameter and 5 mm thick were used as the matrix for fibrovascular tissue growth. The sponge discs were sterilized overnight in 70% ethanol and by boiling in distilled water for 15 min before the implantation. The animals were anesthetized by intraperitoneal injection of 2.5% tribromoethanol (Sigma Chemical Co., St Louis, MO, USA) 1 mL/100 g body weight. The sponge discs were aseptically implanted into a subcutaneous pouch. The animals with implant had been randomly divided into two groups ($n = 10$ each group). Treatment initiated 24 h after the implantation with subcutaneous daily injections of purified recombinant protein (10, 25 or 50 μg per animal per day). The control group received daily injections of 100 μL 0.9% saline. In the eleventh day after the beginning of treatment (ten doses), the implanted bearing mice were anesthetized by intraperitoneal injection of tribromoethanol and killed by cervical dislocation. The sponge was removed, dissected free from adherent tissue, weighed and homogenized for hemoglobin quantitation.

2.10. Hemoglobin measurement

Hemoglobin was quantified by a colorimetric method as described by Santos et al. (2010). Hundred milligrams of the sponge implant were excised carefully. Each piece was homogenized in 2.0 mL Drabkin reagent (Labtest, São Paulo, Brazil) and centrifuged at 10000×g for 15 min. The supernatants were filtered through a 0.22 μm filter (Millipore, Bedfor, MA) and the hemoglobin in the samples was determined spectrophotometrically at 540 nm. The amount of hemoglobin was calculated from a known amount used as standard assayed in parallel. The results were expressed as μg Hb mg^{-1} of wet tissue.

2.11. Statistical analysis

A two-tailed, unpaired Student's t test was done to determine statistical significance by the probability of difference between the means. $p < 0.05$ was considered statistically significant. Values are expressed as mean ± SE.

3. Results

3.1. Cloning of the disintegrin-like

The sequence of the disintegrin-like cDNA presented 279 bp long with the deduced sequence containing 93 amino acids (Fig. 1). The putative primary structure includes 15 cysteine residues and the ECD-motif, the molecular mass was estimated as 10.4 kDa and the isoelectric point 4.1. The protein is 98% homologous to the disintegrin-like segment of jararhagin and 66% homologous to the disintegrin-like segment of leucurolysin-B (leuc-B, Sanchez et al., 2007), an SVMP present in the *B. leucurus* venom (Fig. 2) and therefore was named leucurogin.

3.2. Expression and purification of leucurogin

Leucurogin was successfully expressed by *P. pastoris*. Salts were removed and the protein concentrated using the hollow-fiber system. The protein was purified by one chromatography step process involving ion exchange on

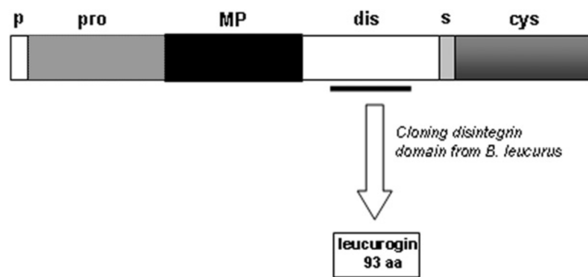

Fig. 1. Leucurogin and the domains of SVMP-III family proteins. These domains include a signal sequence (p), a pro-domain (pro), the metalloproteinase domain (MP), a disintegrin domain (dis), a spacer (s) and the cysteine rich domain (cys).

DEAE-cellulose. Highly purified leucurogin eluted with the buffer containing 200 mM NaCl (Fig. 3A). Fractions containing purified leucurogin were pooled (showed by horizontal line) and loaded on SDS-PAGE. As shown in the Fig. 3B the purified protein presented one band of approximately 10.4 kDa.

3.3. Western blotting

Leucurogin presented 98% homology with jararhagin's disintegrin-like domain. Therefore, we utilized an anti-jararhagin antibody for the characterization of its immunological properties. Leucurogin was recognized by anti-jararhagin antibody (Fig. 4B). As can be seen in Fig. 4A, a second band corresponding to molecular mass of 27 kDa, present in a partially purified fraction of the venom, probably the dis-cys product of hydrolysis of some SVMP from *B. leucurus* venom and a third band from the crude venom (V), corresponding to molecular mass around 60 kDa, probably one native metalloproteinase, were also recognized by that antiserum. Crude venom and P2 are fractions from a purification process described by Sanchez et al. (2007).

3.4. Platelet aggregation

Leucurogin showed to be able to inhibit collagen-induced platelet aggregation but not the one induced by ADP (Fig. 5) or AA. At 0.65 μM leucurogin inhibited 50% of platelet aggregation. At 1.3 μM leucurogin was able to inhibit 100% of platelet aggregation induced by collagen.

3.5. Ehrlich tumor

Tumor mass was evaluated on the 8th day after the beginning of treatment. Leucurogin administration inhibited 30% the tumor growth even at the lower dose of 5 μg/day (0.48 nmol/day) when compared to the control (saline) or with the injection of protein (20 μg/day) from the non-induced fermentation medium (Fig. 6A). Administration of 0.96 nmol (10 μg/day–300 μg/kg/day) of the purified leucurogin significantly inhibited the growth of experimental Ehrlich tumor by more than 50% as compared to the saline (Fig. 6B). The tumor mass from animals treated with 10 μg/day leucurogin was 0.23 ± 0.06 g, and the mass from the group treated with 0.9% saline was 0.49 ± 0.09 g.

3.6. Angiogenesis in sponge disc implant

Angiogenesis was evaluated at day 11 after the beginning of treatment. Neovascularization was also measured

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leuc:        --------------------------------------------------------------------------------
Jar:      1 ATRPKGAVQPKYEDAMQYEFKVNGEPVVLHLEKNKGLFSKDYSEIHYSPDGREITTYPPVEDHCYYHGRIENDADSTASI  80
leuc-B:     --------------------------------------------------------------------------------

leuc:       --------------------------------------------------------------------------------
Jar:     81 SACNGLKGYFKLQRETYFIEPLKLPDSEAHAVFKYENVEKEDEAPKMCGVTQNWKSYEPIKKASQLAFTAEQQRYDPYKY  160
leuc-B:     --------------------------------------------------------------------------------

leuc:       --------------------------------------------------------------------------------
Jar:    161 IEFFVVVDQGTVTKNNGDLDKIKARMYELANIVNEIFRYLYMHVALVGLEIWSNGDKITVKPDVDYTLNSFAEWRKTDLL 240
leuc-B:   1 ---------------------------------------------------------------------------DTVLL 5

leuc:       --------------------------------------------------------------------------------
Jar:    241 TRKKHDNAQLLTAIDFNGPTIGYAYIGSMCHPKRSVGIVQDYSPINLVVAVIMAHEMGHNLGIHHDTGSCSCGDYPCIMG 320
leuc-B:   6 NRISHDNAQLL-AIVFNENVIGKAYTGGMCDPRYSVGVVMDHSPINRLVADTMAHEMGHNLGIHHDTGSCSCGGHSCIMS 84

leuc:     1 -----------------------------------LGTDIISPPVCGNELLEVGEECDCGTPENCQNECCDAATCKLKSG 45
Jar:    321 PTISNEPSKFFSNCSYIQCWDFIMNHNPECIINEPLGTDIISPPVCGNELLEVGEECDCGTPENCQNECCDAATCKLKSG 400
leuc-B:  85 RVISHQPLQYFSNCSYIEYWDFITKLNPQCILNEPLRTDIVSPPVCGNELLEMGEECDCGSPRNCRDLCCDAATCKLHSW 164

leuc:    46 SECGHGDCCEQCKFTKSGTECRASMSECDPAEHCTGQSSECPADVFHK-------------------------------- 93
Jar:    401 SQCGHGDCCEQCKFSKSGTECRASMSECDPAEHCTGQSSECPADVFHKNGQPCLDNYGYCYNGNCPIMYHQCYALFGADV 480
leuc-B: 165 VECESGECCDQCRFIKAGNVCRPPRKECDVAEACTGQSAQCPTDDFKRNGQPCLNNYAYCYQGNCPIMYHQCYALFGSDA 244

leuc:       --------------------------------------------------------------------------------
Jar:    481 YEAEDSCFKDNQKGNYYGYCRKENGKKIPCAPEDVKCGRLYCKDNSPGQNNPCKMFYSNDD--EHKGMVLPGTKCADGKV 558
leuc-B: 245 TMAQDSCFQVNKKGNEYFYCRLENGINIPCAQEDVKCGRLFCHN----------MKYEQDCNYSDRGMVDNGTKCAEGKV 314

leuc:       ---------------
Jar:    559 CSNGHCVDVATAY - 571
leuc-B: 315 CNSNR-----QAYQR 324
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Fig. 2. Amino acid alignment. Sequences of leucurogin (leuc), jararhagin (jar, Paine et al., 1992), and leucurolysin-B (leuc-B, Sanchez et al., 2007) showing disintegrin sequences (bold). Amino acids not homologous with jararhagin are underlined.

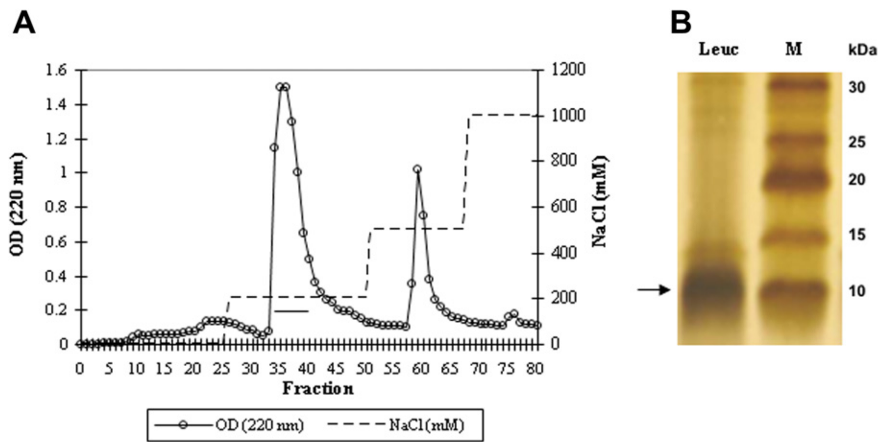

Fig. 3. Leucurogin purification. A, Profile obtained in DEAE-cellulose (2 × 3 cm). Fractions of 2 mL were collected. The horizontal bar refers to the active pooled fractions. B, Polyacrylamide gel electrophoresis (12%) under reducing conditions of leucurogin (Leuc) eluted from ionic exchange (200 mM) chromatography. The gel was silver stained. The arrow indicates the leucurogin band. M, molecular mass marker (Bench marker ladder, Invitrogen). OD = optical density.

by evaluating the amount of hemoglobin within the sponge. There was a significant decrease (~82%) in the hemoglobin levels in the sponge of animals treated with 10 μg/day of leucurogin and at 50 μg/day the decreasing was around 100% (Fig. 7).

4. Discussion

Bothrops snake venoms are rich sources of metalloproteinases, enzymes involved in the hemorrhagic process caused by the venom Bjarnason and Fox (2004). These proteinases, by autolysis, may generate some

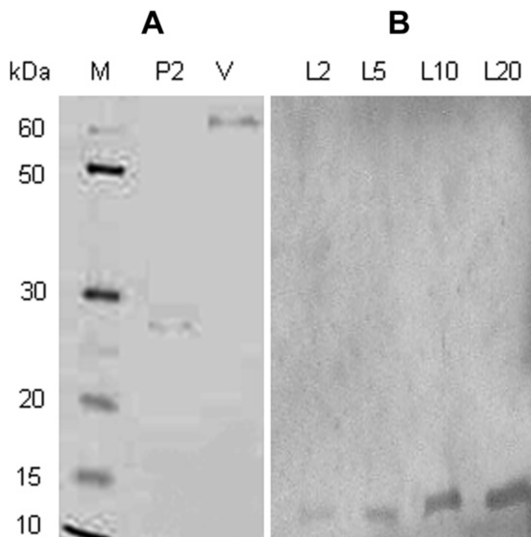

Fig. 4. Western-blot analysis. Samples were electrophoresed in 12% polyacrylamide gels and electroblotted to PVDF membranes. A polyclonal antibody anti-jararhagin was used for detection. This gel is representative of 3 experiments with similar results. A) V, crude venom containing native metalloproteinases of *B. leucurus*, P2 - fraction from *B. leucurus* venom (Sanchez et al., 2007), M - molecular mass marker (Bench marker ladder, Invitrogen) and B) leucurogin (L) 2, 5, 10 or 20 μg.

bioactive fragments known as disintegrins or the conjugate dis-cys depending of the snake species (Takeda et al., 2006). A growing body of evidences showing the ability of disintegrins to inhibit platelet aggregation and its effects involving the largely distributed membrane receptors integrins has been accumulated in the literature. It was observed in our lab that one proteinic fraction, partially purified from *B. leucurus* venom, is able to inhibit tumor growth implanted in mice. This fraction, presenting a 27 kDa protein is able to inhibit Ehrlich tumor growth by 60% when subcutaneously injected in the mice at 300 μg/kg body weight/day during 9 days (unpublished data). We believed that the effect upon tumor growth was due to the 27 kDa protein, probably one dis-cys conjugate. As the biological effects of dis-cys conjugate are not well defined, if attributed to the disintegrin-like or to the cysteine rich domain, we decided, for a better biological characterization, to produce the recombinant disintegrin-like segment. Recombinant DNA techniques gives us the possibility to obtain, in large amounts, proteins not found in nature in a free form, allowing the study of their putative biological properties, therefore providing pivotal tools to understand different biological processes.

Recent studies have examined the participation of integrin–disintegrin interaction in physiological and pathophysiological processes (Takeda et al., 2006; Kamiguti et al., 1998; Clemetson, 1998). Due to their ability to inhibit adhesion, disintegrins may represent potential tools for cancer therapy since adhesion is an important step for angiogenesis development. Jararhagin C, a 30 kDa dis-cys hydrolysis product of jararhagin (Moura da Silva et al., 1999; Usami et al., 1994) and halydin, the firstly described recombinant disintegrin-like (You et al., 2003), are potent inhibitors of platelet aggregation. Leucurogin, the ECD recombinant disintegrin-like described in this study showed to be active against tumor growth. Leucurogin is 66% homologous to the disintegrin-like segment of leucurolysin-B, one SVMP class III isolated from *B. leucurus* venom (Sanchez et al., 2007). This result shows that the

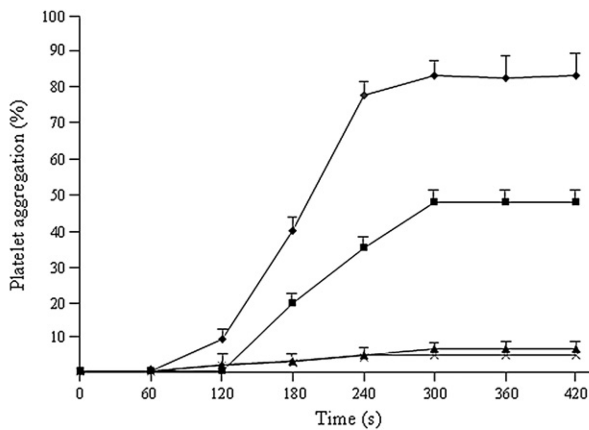

Fig. 5. Effect of leucurogin on platelet aggregation. Platelets-rich plasma were pre-incubated at 37 °C for 2 min without (◆) and in the presence of leucurogin at concentrations 0.65 μM (■) and 1.3 μM (▲) before the addition of 5 μg/mL collagen. (x) control, without collagen as an agonist of the process. The experiments were repeated at least three times.

venom of *B. leucurus*, and probably also from other species, contains more than one type of dis-cys conjugate.

Leucurogin used in the biological assays in this study was purified by a very simple procedure involving one chromatographic step after clarification in a hollow-fiber system. As observed for most recombinant proteins, leucurogin has a strong tendency to aggregate in low ionic strength (data not shown). Purified leucurogin was firstly assayed for inhibition of platelet aggregation and the results showed that the recombinant protein is as active as the other natural disintegrins or dis-cys conjugates like that from *B. jararaca* (Usami et al., 1994) and *Bothrops atrox* (Jia et al., 1997). At micromolar levels leucurogin is able to inhibit 100% of platelet aggregation induced by collagen. No effects were observed upon platelet aggregation induced by ADP or AA. The capacity of leucurogin to inhibit the growth of Ehrlich tumor implanted in mice was also similar to that observed for the 27 kDa protein partially purified from *B. leucurus* snake venom. By the vascularization levels of a sponge subcutaneously implanted in mice we can conclude that at least partially the effect of leucurogin upon the tumor growth may be due to a potent inhibition of angiogenesis process. Previous studies have shown that hemoglobin detection correlated well with other methods for the detection and quantification of angiogenesis in tissues (Hu et al., 1995).

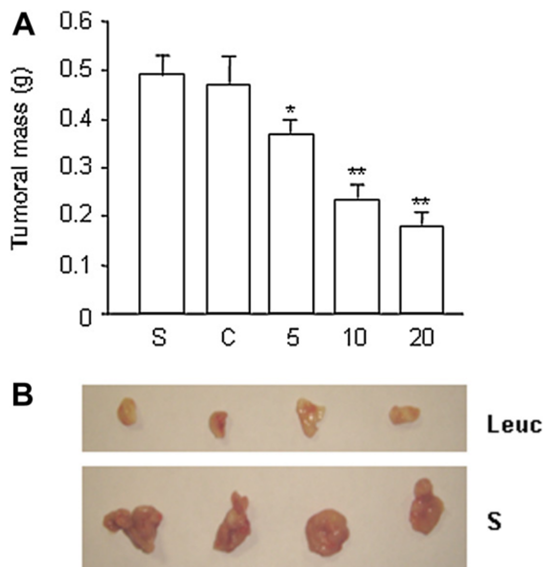

Fig. 6. Effect of leucurogin upon tumor growth. A) Tumor mass in male Swiss mice inoculated with Ehrlich tumor. Animals were divided in groups and treated with disintegrin with daily injections of 5, 10 or 20 μg/day for seven days. In the animals from control group it was injected 100 μL of saline (S). For fermentation control it was used a sample of fermentation medium without disintegrin (C) in a dose of 20 μg protein/day. *$p < 0.05$ *vs* S. **$p < 0.01$ *vs* saline. B) Illustrations of tumors: animals treated with saline (S) or with 20 μg per day of leucurogin (Leuc).

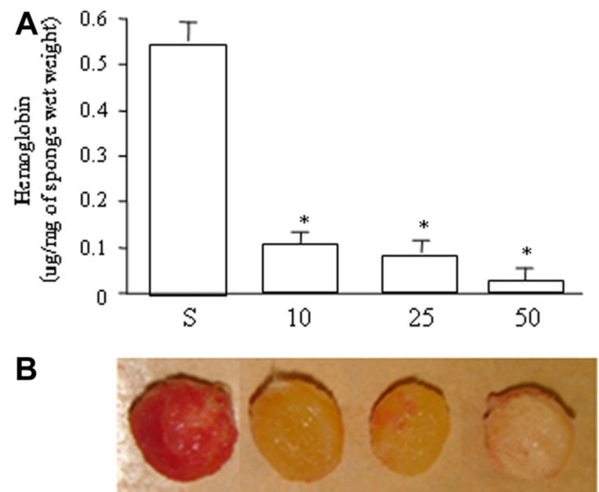

Fig. 7. Angiogenesis level in implanted sponge evaluated in the 11th day after beginning of treatment with saline (S) or with 10, 25 or 50 μg of leucurogin/animal/day). A) hemoglobin levels determined colorimetrically and expressed as microgram per milligram of wet weight of sponge. B) Illustrations of sponges after treatment. Results represent the mean ± SEM of ten animals for each group. *$p < 0.05$ when compared to control (S).

In conclusion, this work describes, for the first time, the production of one recombinant disintegrin-like cloned from *B. leucurus* and shows that this disintegrin, independently of the cysteine rich domain, is able, probably through interaction with integrins $\alpha_1\beta_1$ or $\alpha_2\beta_1$, to inhibit effects elicited by type I collagen like platelet aggregation and tumor growth. Leucurogin represents a new tool to understand the biological process where disintegrins-like are involved and may help to characterize integrins that can be involved in development and progression of malignant cells.

Conflicts of interest

None.

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