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Antithrombotic effect of *Lonomia obliqua* caterpillar bristle extract on experimental venous thrombosis

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Abstract

The venom of *Lonomia obliqua* caterpillar may induce a hemorrhagic syndrome in humans, and blood incoagulability by afibrinogenemia when intravenously injected in laboratory animals. The possible antithrombotic and thrombolytic activities of *L. obliqua* caterpillar bristle extract (LOCBE) were evaluated in this study. The minimal intravenous dose of the extract necessary to induce afibrinogenemia and anticoagulation was 3.0 and 10.0 µg protein/kg body weight for rabbits and rats, respectively. In rabbits, this dose induced total blood incoagulability for at least 10 h and did not reduce the weight of preformed venous thrombi, in contrast to streptokinase (30,000 IU/kg). In rats, pretreatment with 5.0 and 10.0 µg/kg LOCBE prevented the formation of thrombi induced by venous stasis or by injury to the venous endothelium. The dose of 5.0 µg/kg LOCBE did not modify blood coagulation assay parameters but increased bleeding time and decreased plasma factor XIII concentration. When the extract was administered to rats at the dose of 10.0 µg/kg, the blood was totally incoagulable for 6 h. These data show that LOCBE was effective in preventing experimental venous thrombosis in rats, justifying further studies using purified fractions of the extract to clarify the mechanisms of this effect.

Key words
- Thrombosis
- Fibrinolysis
- Anticoagulant
- Venom
- *Lonomia obliqua* caterpillar

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Introduction

Many natural substances derived from invertebrates have toxic effects on the blood coagulation and fibrinolytic systems of mammals (1). Some of these substances have been well characterized and studied as alternative drugs in the treatment of pathological processes that affect the hemostatic system. Hirudin (2) and tick anticoagulant peptide (3) are examples of substances which have been studied both experimentally and clinically as possible antithrombotic drugs because of their anticoagulant activity *in vivo*.

Cases of acquired hemorrhagic diathesis in humans due to accidental contact with *Lonomia* caterpillar bristles (*L. achelous* and *L. obliqua*) have been reported in South America. Symptoms of acute envenoming by these two caterpillars consist of local pain, ecchymosis, headache, acute renal failure and hemorrhagic episodes. These episodes, which sometimes can be fatal, affect mucous membranes, lungs, intraperitoneal cavity and the central

nervous system or medullary canal. *L. obliqua* (Walker) is found mainly in the southern states of Brazil and *L. achelous* in Venezuela.

Arocha-Piñango et al. (4,5) have published several studies on aspects of *L. achelous* envenomation, common laboratory findings in envenomed patients (6,7), and on the effects of the venom and fractions derived from the venom on coagulation and fibrinolysis (8-12).

L. obliqua caterpillar bristle extract (LOCBE) induces *in vitro* calcium-dependent procoagulant activity both in human and rat plasma (13), probably due to the activation of prothrombin and factor X, with no effects on platelets (14). *In vivo*, it causes blood incoagulability in laboratory animals (15,16). Recently, an *L. obliqua* prothrombin-activator serine-protease was isolated from LOCBE and characterized in our laboratory (16).

Since LOCBE induces blood incoagulability, the aim of the present study was to investigate its effects in preventing thrombus formation in the vena cava and jugular vein of rats, and a possible lytic action on preformed thrombus in the jugular vein of rabbits, as an initial screening for further studies.

Material and Methods

Animals

Male Wistar rats (280-350 g) and male New Zealand rabbits (3.0-4.2 kg) were bred at Instituto Butantan, São Paulo, SP, Brazil. The animals had free access to water and food and were on a 12-h light-dark cycle. Procedures involving animals and their care were conducted in accordance with the Guidelines for the Use of Animals in Biochemical Research (17).

Drugs

The drugs used were streptokinase (Carlo Erba, São Paulo, SP, Brazil), sodium pentobarbital (a gift from Abbott do Brasil, São Paulo, SP, Brazil), sodium heparin (Liquemine, Roche Pharmaceuticals, São Paulo, SP, Brazil), bovine thrombin (Hoffman-La Roche, Basel, Switzerland), thromboplastin (Baxter Diagnostic Inc., Deerfield, IL, USA), sodium iodoacetate (Aldrich Chemical Company, Inc., Milwaukee, WI, USA), factor XIII reagents (Diagnostica Stago, Asnières, France), urea and rabbit brain cephalin (Sigma, St. Louis, MO, USA), and human fibrinogen (Chromogenix, Mölndal, Sweden).

Preparation of the *L. obliqua* caterpillar bristle extract

L. obliqua were collected in rural areas of Rio Grande do Sul and Santa Catarina and maintained in the Laboratory of Entomology of Instituto Butantan. Preparation of a crude LOCBE has been standardized (15). Fifteen caterpillars were anesthetized under a CO_2 atmosphere, the bristles were cut off with scissors at their insertion in the tegument and suspended (10%, w/v) in cold phosphate-buffered saline, pH 7.4. This suspension was homogenated and centrifuged at 2100 *g* for 10 min at 4ºC. The supernatant was collected and its protein content was determined using a modification of the Lowry method (18). Supernatant aliquots were stored at -70ºC and the same batch was used in all experiments.

Antithrombotic activity of LOCBE injected into the vena cava and jugular vein of rats

Determination of LOCBE doses for intravenous (iv) administration in rats. Previous studies have found that the minimal dose of LOCBE needed to incoagulate the blood (MDIB) after intradermal injection in rats was 140 µg/kg (15). For the determination of MDIB for *iv* administration, the rats were anesthetized with sodium pentobarbital (60

mg/kg, intraperitoneally, *ip*). LOCBE infusions (1 ml) were administered over a period of 30 min into the cannulated right jugular vein at doses that varied from 2.5 to 140.0 µg/kg for each group of 4 animals. Thirty minutes after the end of the infusion, 1 ml of blood was collected from the carotid artery with a siliconized needle for the measurement of whole blood clotting time (WBCT). WBCT was determined by measuring the clotting time of 1 ml of venous blood in a glass test tube at 37ºC (19). The MDIB obtained was 10.0 µg/kg. The duration of the anticoagulant effect of LOCBE was determined in these experiments using doses corresponding to 0.5, 1 and 2 MDIB and by measuring WBCT at 0.5, 2, 5, 10, and 24 h after the end of infusion. Blood samples were also collected from control animals treated with saline. The rats were randomly allocated to groups of 4 animals each.

Prevention of thrombus formation in rats

Prevention of thrombus formation by treatment with LOCBE was tested in two different models of experimental thrombosis: one caused mainly by stasis in the vena cava, and the other by endothelium injury in the jugular vein. For both experiments, rats were anesthetized with sodium pentobarbital (60 mg/kg, *ip*) and randomly allocated to one of the following groups of 8 animals each: control (saline), 300 IU/kg heparin, and 2.5, 5.0 and 10.0 µg/kg LOCBE. Drugs (1 ml/30 min) were infused into the left jugular vein 1 h before thrombus induction.

The technique described by Reyers et al. (20) was used for stasis-induced thrombus formation in the vena cava. Briefly, the abdomen was opened with a midline incision, the vena cava isolated, and all branches between the left renal vein and iliac veins were tied. The vena cava was tied just caudally to the left renal vein and the incision closed. Four hours later, the animals were re-anesthetized, the abdominal incision was reopened and the vena cava segment removed. When the thrombus was present, it was removed from the segment, blotted on filter paper, and weighed both immediately (wet weight) and after drying at 37ºC for 24 h (dry weight).

The technique described by Maffei et al. (21), with modifications in the solution used and in the way of injection, was used for injury-induced thrombus formation in the jugular vein. Briefly, the right jugular vein was exposed through a midline incision in the neck. The vein was cleared and a segment was isolated with two cotton thread loops placed 1 cm apart. The segment had the blood removed through an internal jugular vein tributary and was then filled with 50% glucose. Ten minutes later, the blood flow was re-established by removal of the loops, the internal jugular vein was tied, and the incision closed. Three hours later, the animals were re-anesthetized, the vein segment was removed, and when present, the thrombus was collected and weighed as described previously.

Blood samples (2 ml) were collected from the carotid arteries before drug infusion, thrombus induction, and vein removal. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured according to standardized techniques described by Denson (19), with standardized reagents. To measure thrombin time (TT), 100 µl of thrombin (6 IU/ml) was added to 200 µl of plasma and the clotting time recorded at 37ºC (22). Fibrinogen was estimated by the method of Ratnoff and Menzie (23). Bleeding time (BT) was also measured by making a small incision (3 mm in length, 2 mm in depth) 9 cm from the tail end. The tail was immersed in 0.9% saline at 37ºC, and the time of bleeding cessation was recorded (24).

Effect of LOCBE on whole blood lysis time and plasma factor XIII activity

To study the *in vivo* effect of LOCBE on

plasma factor XIII activity, LOCBE was infused *iv* at the dose of 5.0 µg/kg, and WBCT, whole blood lysis time (WBLT) and plasma factor XIII activity were measured. Blood was collected from 8 rats 5 min before (control) and 30 min after LOCBE infusion. WBCT was determined by measuring the clotting time of 1 ml of venous blood in a glass test tube at 37ºC (19), and WBLT by measuring the time needed for clot lysis. Factor XIII zymogen concentration in plasma samples was determined using the sodium iodoacetate tolerance test (25). Briefly, plasma clots were formed by recalcification of plasma samples (100 µl of 50 mM $CaCl_2$ + 200 µl of plasma) in the presence of 100 µl of sodium iodoacetate at different concentrations (from 5 to 35 mM). After incubation for 30 min at 37ºC, 3 ml of 5 M urea was added and time for clot dissolution was measured. The control plasma was diluted to the same fibrinogen concentration as the tested plasma.

Evaluation of LOCBE thrombolytic activity on preformed thrombus in rabbits

The MDIB for *iv* administration in non-anesthetized rabbits, determined by the same procedure as used in rats, was 3.0 µg/kg. Drugs were infused through the marginal vein of one ear, and blood samples were collected from the marginal vein of the contralateral ear.

Thrombi were induced using the technique of Rollo et al. (26). Briefly, rabbits were anesthetized by *iv* injection of sodium pentobarbital (30 mg/kg). A 5-cm segment of the right jugular vein (above the junction of the internal and external jugular veins) was dissected free of surrounding tissue and all side branches were tied. Three semi-occlusive cotton ligatures 1.2, 1.0 and 0.8 mm in diameter were placed around the jugular vein 2 mm apart, with the help of three different needles. The stenosis with the smaller diameter was placed closer to the heart, and a funnel-like structure was formed. Two cotton thread loops were placed above the funnel-like structure in order to isolate a 2-cm long segment. Blood in this segment (about 0.5 ml) was collected from a small side branch into a syringe containing 30 U of thrombin, and 50 µl of this mixture was re-injected through this branch into the vein. After injection, the side branch was tied and the blood allowed to clot. Ten minutes later, both vessel loops were removed and blood flow was re-established. The formed thrombus was trapped in the funnel but did not block the blood flow, remaining trapped until the beginning of drug infusion.

Different doses of LOCBE (1.5, 3.0 and 4.5 µg/kg) were infused for evaluation of its possible thrombolytic activity. The doses were infused through the marginal vein (30 ml/2 h) starting 30 min after loop removal. The rabbits in one control group received only vehicle (isotonic saline), and those in the other group received streptokinase (30,000 IU/kg). The rabbits were randomly allocated to these treatment groups of 8 animals each.

Three hours after drug infusion, the animals were re-anesthetized and the venous segment was removed. The thrombus was removed from the segment, blotted on filter paper and weighed both immediately (wet weight) and after drying for 24 h at 37ºC (dry weight). Blood samples (3.0 ml + 3.8% sodium citrate 1:10 (v/v)) were collected from the marginal vein of the contralateral ear 15 min before thrombus induction and before thrombus evaluation. These samples were centrifuged for 10 min at 2000 *g* to obtain plasma and kept refrigerated until analysis on the same day. The following tests were performed: PT, APTT, TT, fibrinogen concentration, and fibrinolytic activity of the euglobulin fraction.

Coagulation and fibrinolysis assays

PT, APTT, TT and fibrinogen concentra-

tion were measured as described above. For determination of fibrinolytic activity, the euglobulin fraction was prepared with 200 µl of plasma diluted in 1.8 ml of cold water, precipitated with 150 µl of 0.25% acetic acid (27) and resuspended in 200 µl of 100 µM 0.1% Tris Tween buffer, pH 7.4 (28). The fibrinolytic activity of the euglobulin fraction was measured using the fibrin-agarose plate method (29). Fibrin-agarose plates were prepared with a 1/1 mixture of 2.0 mg/ml of human fibrinogen (Chromogenix) and 2.0% agarose (Sigma) diluted in 100 mM sodium pentobarbital buffer, pH 7.75, containing 1.66 M $CaCl_2$, 0.68 mM $MgCl_2$, and 93.3 mM NaCl. Six milliliters of this mixture was placed on plastic dishes 9 cm in diameter and clotted with 4 NIH U of bovine thrombin. Three to six samples of 30 µl of euglobulin fractions were applied to each plate. After 18 h at 37ºC, lysis areas (mm^2) were calculated as the product of two perpendicular diameters. The results are reported as means ± SD for N = 8 in each experimental group.

Statistical analysis

The results are reported as means ± SD. The results of coagulation tests (TT, APTT, PT, BT) and fibrinolytic activity (lysis area on fibrin-agarose plates) before and after treatment were analyzed by the Student paired *t*-test. Analysis of variance (ANOVA) for completely randomized groups was used for the evaluation of differences between groups. The incidence of thrombi in the drug-treated groups was compared to the control group (isotonic saline) by the Fisher exact test. Factor XIII activity was compared before and after LOCBE treatment by the non-parametric Wilcoxon test. Differences between groups were compared by the Kruskal-Wallis test (30). The differences were considered significant when $P<0.05$.

Results

Determination of the minimum doses of LOCBE needed to incoagulate blood

The MDIB by *iv* LOCBE infusion in rats was 10.0 µg/kg. This dose induced blood incoagulability for at least 10 h with return of the WBCT to normal values before 24 h.

Evaluation of LOCBE antithrombotic activity in vena cava and jugular vein of rats

Vena cava. All animals of the control group treated with saline (N = 8) and of the LOCBE group treated with 2.5 µg/kg (N = 8) presented thrombi. The frequency in each group is presented in Table 1. LOCBE at the dose of 5.0 µg/kg prevented

Table 1. Effect of *Lonomia obliqua* caterpillar bristle extract (LOCBE) and heparin (300 IU/kg) on the frequency and weight of thrombi induced in the vena cava and jugular vein of rats.

Groups	Vena cava			Jugular vein		
	T/n	WW	DW	T/n	WW	DW
Saline	8/8	19.75 ± 3.37	5.91 ± 0.92	8/8	20.88 ± 2.92	8.50 ± 1.34
Heparin	2/8*	6.50 ± 0.71	2.25 ± 0.21	3/8*	9.90 ± 2.87	3.94 ± 1.02
LOCBE (µg/kg)						
2.5	8/8	20.00 ± 3.42	6.02 ± 1.52	8/8	21.05 ± 2.67	8.75 ± 1.56
5.0	1/8*	11.00	4.58	2/8*	9.60 ± 4.23	3.81 ± 1.56
10.0	0/8*	0	0	0/8*	0	0

Values are reported as mg (means ± SD). N = 8 animals in each group. WW, wet weight; DW, dry weight; T/n, number of animals with thrombus.
*P<0.05 for each treatment group compared to control (paired *t*-test).

thrombus formation in 7 of the 8 animals, without significant differences in coagulation assay values when compared with pre-infusion values. LOCBE at the dose of 10.0 µg/kg inhibited thrombus formation in all treated animals and induced total incoagulability (Table 2).

The lower doses of LOCBE (2.5 and 5.0 µg/kg) did not significantly change TT, PT, or APTT when compared with pre-infusion values. However, 5.0 µg/kg LOCBE increased BT from 114.25 ± 1.22 to 141.00 ± 2.60 s at 30 min and to 139.75 ± 1.53 s at the end of the experiment ($P<0.05$). The highest dose of LOCBE (10.0 µg/kg) increased BT from 109.62 ± 1.96 to 261.37 ± 10.37 s, 30 min after the end of the infusion, and to 257.50 ± 8.72 s at the end of the experiment (Table 2).

Heparin (300 IU/kg) prevented thrombus formation in 6 of the 8 treated animals, and caused a significant increase in TT, PT, APTT and BT assays in all animals (Table 2). Significant reduction of thrombus mean weight was observed in the two other animals (Table 1).

Thirty minutes after infusion, LOCBE reduced the plasma fibrinogen concentrations to 75% of the mean control value at 5.0 µg/kg and to undetectable values at 10.0 µg/kg (N = 8) (Table 3).

Jugular vein. The injection of 50% glucose solution into the jugular vein segment of rats led to the development of an occlusive thrombus in 100% of the animals treated with saline (control group) and with 2.5 µg/kg LOCBE. Heparin (300 IU/kg) and 5.0 µg/kg LOCBE (N = 8 for each treatment group) prevented thrombus formation in 5 and 6 of the 8 treated animals, respectively. Thrombus mean weight in the remaining animals was significantly reduced (Table 1).

The effects of LOCBE (2.5, 5.0 and 10.0 µg/kg) and heparin (300 IU/kg) treatments

Table 2. Effects of heparin and *Lonomia obliqua* caterpillar bristle extract (LOCBE) infusion on the coagulation parameters of rats.

Compound	Assays			
	TT	PT	APTT	BT
Heparin (300 IU/kg)				
-5 min	16.62 ± 0.50	13.50 ± 0.68	19.25 ± 0.82	103.62 ± 2.84
+30 min	24.25 ± 0.96*	25.12 ± 1.37*	37.87 ± 1.01*	132.37 ± 1.52*
+240 min	25.50 ± 0.68*	23.75 ± 1.16*	41.62 ± 1.19*	129.75 ± 3.60*
LOCBE (2.5 µg/kg)				
-5 min	17.25 ± 0.88	13.12 ± 0.58	17.87 ± 1.03	115.00 ± 3.51
+30 min	15.87 ± 0.87	12.25 ± 0.37	17.86 ± 0.91	112.87 ± 2.12
+240 min	18.25 ± 0.75	13.50 ± 0.57	17.62 ± 0.80	108.62 ± 2.99
LOCBE (5.0 µg/kg)				
-5 min	18.25 ± 0.75	12.75 ± 0.45	19.25 ± 0.88	114.25 ± 1.22
+30 min	17.62 ± 0.60	12.50 ± 0.42	20.00 ± 0.65	141.00 ± 2.60*
+240 min	17.75 ± 0.65	14.00 ± 0.46	21.00 ± 1.13	139.75 ± 1.53*
LOCBE (10.0 µg/kg)				
-5 min	18.37 ± 0.60	13.00 ± 0.40	19.00 ± 0.71	109.62 ± 1.96
+30 min	Inc*	Inc*	Inc*	261.37 ± 10.37*
+240 min	Inc*	Inc*	Inc*	257.50 ± 8.72*

Blood samples were collected 5 min before (-5), 30 and 240 min after drug infusion. Values are reported as seconds (mean ± SD). N = 8 animals in each group. TT, thrombin time; PT, prothrombin time; APTT, activated partial thromboplastin time; BT, bleeding time; Inc, incoagulable.
*$P<0.05$ for each treatment group compared to control -5 min (paired *t*-test).

on coagulation assays, plasma fibrinogen concentration, and BT were similar to those found in the vena cava experiments.

Effects of 5.0 µg/kg LOCBE on whole blood lysis time and plasma factor XIII

WBLT of samples collected 30 min after LOCBE infusion (5.0 µg/kg) decreased significantly (from >24 to <2 h) in all animals (N = 8). Clots formed in plasma in the presence of all sodium iodoacetate concentrations were dissolved in 5 M urea within less than 90 min. Control plasma clots were insoluble after 6 h (P<0.001) (Wilcoxon test).

Determination of LOCBE doses for *iv* administration to rabbits

The MDIB for *iv* LOCBE infusion in rabbits was 3.0 µg/kg. This dose induced blood incoagulability for at least 7 h, with WBCT returning to normal levels within 24 h.

Evaluation of LOCBE lytic activity on preformed venous thrombus in rabbits

Thirty minutes after thrombus induction, drugs were infused *iv* in all animals (N = 8, for each treatment group). All saline-treated rabbits showed non-occlusive thrombi with a mean wet and dry weight of 128.87 ± 16.26 and 41.62 ± 5.83 mg, respectively. Streptokinase (30,000 IU/kg) induced total thrombus lysis in 6 of the 8 animals and significantly decreased the mean thrombus weight in the 2 remaining animals (19 ± 1.41 and 7.10 ± 0.14, wet and dry weights, respectively). Even at doses that produced total incoagulability and higher plasma fibrinolytic activity, LOCBE did not reduce the frequency or mean weight of venous thrombi present in these animals (groups treated with LOCBE at 1.5, 3.0 and 4.5 µg/kg). The result obtained with streptokinase was statistically different from control and the three doses of LOCBE (paired *t*-test).

Coagulation and fibrinolysis assays

Treatment with LOCBE (3.0 and 4.5 µg/kg) (N = 8 for each treatment group) reduced plasma fibrinogen concentrations to undetectable values and induced total incoagulability. The lowest dose of LOCBE (1.5 µg/kg) did not significantly change APTT, TT, or PT when compared to control animals that received saline infusion (N = 8).

Streptokinase (30,000 IU/kg) increased TT by 73% (P<0.001) and PT by 12.7% in comparison with pre-infusion values. In addition, streptokinase reduced plasma fibrinogen concentration by 30% (from 276 ± 43 to 192 ± 38 mg%, P<0.001).

Increases in lysis areas on fibrin-agarose plates (pre- *vs* post-infusion values) produced by euglobulin fractions from 8 animals treated with streptokinase were significantly higher than those of LOCBE-treated groups. In the streptokinase-treated group, the mean increase was 77% (from 153 ± 7 to 272 ± 12 mm^2, P<0.001), while in the LOCBE-treated groups, increases were of 17% at 3.0 µg/kg (from 159 ± 17 to 186 ± 13 mm^2, P = 0.004) and 20% at 4.5 µg/kg (from 172 ± 15 to 206 ± 19 mm^2, P = 0.002) (Table 4).

Discussion

The present study on the thrombolytic effect of LOCBE was carried out in rabbits,

Table 3. Mean fibrinogen concentration in rat and rabbit plasmas after injection of *Lonomia obliqua* caterpillar bristle extract (LOCBE).

Animal	Pre-infusion value	Hours after infusion		
		0.5	2	4
Rats				
5.0 µg/kg	540.6 ± 27.8	406.8 ± 17.4*		397.7 ± 57.8*
10.0 µg/kg	552.3 ± 26.3	0*		0*
Rabbits				
3.0 µg/kg	263.0 ± 8.8		0*	

Values are reported as mg% (means ± SD). N = 8 animals in each group. 0, not found. *P<0.05 compared to control (pre-infusion values; paired *t*-test).

since this model of venous thrombosis is highly reproducible and has been used by others with a good thrombolytic response to agents such as streptokinase (31). Non-occlusive thrombi inside the rabbit jugular vein were formed 10 min after induction and remained in place up to 5 h in 100% in the control group animals. Streptokinase induced both thrombolysis and an increase in fibrinolytic activity. LOCBE infusion did not cause thrombolysis at the doses used, but slightly increased fibrinolytic activity. This fibrinolytic activity detected after LOCBE infusion may have been caused by secondary activation of fibrinolysis, not sufficient to cause local thrombolysis.

Induction of thrombosis by venous stasis in the vena cava or by endothelial injury in the jugular vein of rats induced thrombus formation in all control animals. This model of thrombosis induction by vena cava ligature was chosen because it is simple, sensitive and highly reproducible. In addition, one antithrombotic drug of reference (heparin) can be used (32). The other method for venous thrombosis induction by injection of hypertonic glucose was used to study possible LOCBE antithrombotic activity on thrombi formed due to endothelial injury.

In these models, *iv* infusion of LOCBE at the dose of 10.0 µg/kg completely prevented thrombus formation, inducing blood incoagulability by afibrinogenemia and increasing BT by 2.4-fold. These alterations were reversed within 24 h. LOCBE at 5.0 µg/kg also prevented thrombus formation in the jugular vein and vena cava in 6 and 7 of the 8 tested animals, respectively. This dose produced a mean 25% reduction of plasma fibrinogen concentration, preserving normal coagulation assay values, but increasing BT 1.2-fold. At this dose, LOCBE showed antithrombotic activity similar to that obtained with heparin, with fewer alterations in coagulation assays. Also, blood samples collected from LOCBE-treated animals (5.0 µg/kg) 30 min after infusion showed WBLT <2 h, suggesting an activation of the fibrinolytic system or an increased clot susceptibility to plasmin activity. When plasma from these

Table 4. Effects of streptokinase and *Lonomia obliqua* caterpillar bristle extract (LOCBE) infusion on the coagulation parameters of rabbits.

Compound	Assays			
	TT	PT	APTT	Lysis
SK (30,000 IU/kg)				
-15 min	15.6 ± 2.0	16.3 ± 3.2	59.2 ± 3.2	153.7 ± 7.8
+180 min	25.6 ± 2.8*	18.7 ± 2.3	56.5 ± 4.8	272.3 ± 12.5*
LOCBE (1.5 µg/kg)				
-15 min	14.1 ± 2.6	15.0 ± 2.0	56.2 ± 5.7	167.8 ± 20.2
+180 min	14.8 ± 2.0	14.7 ± 3.2	56.6 ± 5.7	169.3 ± 39.7
LOCBE (3.0 µg/kg)				
-15 min	14.7 ± 2.2	14.8 ± 3.1	55.7 ± 6.3	159.2 ± 17.3
+180 min	Inc*	Inc*	Inc*	186.0 ± 13.0
LOCBE (4.5 µg/kg)				
-15 min	14.2 ± 2.1	12.8 ± 2.4	56.2 ± 5.4	172.5 ± 15.5
+180 min	Inc*	Inc*	Inc*	206.7 ± 19.7*

The animals received streptokinase (SK, 30,000 IU/kg) or LOCBE at the doses of 1.5, 3.0 and 4.5 µg/kg. Blood samples were collected 15 min before (-15) and 180 min after drug infusion. Values are reported as seconds (mean ± SD). N = 8 animals in each group. TT, thrombin time; PT, prothrombin time; APTT, activated partial thromboplastin time; Lysis area (mm^2); Inc, incoagulable.
*P<0.05 for each treatment group compared to control (paired *t*-test).

blood samples was incubated with sodium iodoacetate and then recalcified, the resulting clots were dissolved within less than 90 min (control >24 h) after re-suspension in 5 M urea, suggesting a low concentration or low activity of factor XIII. The decrease in factor XIII level or activity reduces cross-linking of α_2-antiplasmin to fibrin, amplifying the action of plasmin (33).

Despite some difficulty in interpreting the effects of crude extracts on hemostasis, at least part of the anticoagulant and antithrombotic effects observed in our experiments is probably a consequence of fibrinogen consumption, as reported for a number of substances isolated from snake venoms, such as ancrod, batroxobin and crotalase. However, the mechanism of action of these snake enzymes seems to be different, as they provoke a direct cleavage of fibrinopeptide A from fibrinogen, without activation or consumption of factor XIII (34,35). Although it has been shown that LOCBE activates factor X (14) and a fraction that activates prothrombin has been isolated and characterized *in vivo* (16,36), the intrinsic mechanism of the hypofibrinogenemia induced by LOCBE is not completely understood and needs further investigation.

The decreased level of plasma factor XIII zymogen in treated animals in our experiments seems to be similar to that observed in human accidents with *Lonomia* caterpillars (37). However, this decrease in plasma factor XIII in plasma of envenomed patients, after contact with *L. achelous* and *L. obliqua* caterpillars seems to be a consequence of different mechanisms. The venom of *L. achelous* contains one well-characterized substance that degrades human factor XIII *in vitro* (10-12,37). This substance, called Lonomin V, produces a dose-dependent thrombolysis in combination with a decrease in factor XIII level (37). To date, however, no similar substance was described in *L. obliqua* venom. The differences in the *in vitro* activity of *L. obliqua* and *L. aquelous* toxins could be due to species differences or different caterpillar materials. Tests with *L. aquelous* have been mainly performed with hemolymph, while tests with *L. obliqua* were performed with bristle extracts (37). One possible explanation for the low level of factor XIII in the treated rats in our experiments could be consumption, as observed in other types of disseminated intravascular coagulation (38).

The mechanism of action of the significant increase of BT seen in LOCBE-treated rats is unexplained, and deserves further study. The explanation could be alterations in platelet number or function or in vascular reactivity and the increase in BT could be induced by the antithrombotic components or by other substances present in this crude extract.

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