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Regional Blood Flow after Intravenous Administration of Scorpion Venom in Rats

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Abstract

The accidents by scorpion stings in Brazil, particularly in Belo Horizonte, capital of Minas Gerais state are caused by *Tityus serrulatus* species and accounts for fatal stings, especially in children and elderly people. The death in severe evenomation is caused by a circulatory shock, associated with respiratory and cardiovascular arrhythmias, cardiogenic shock, pulmonary edema, respiratory depression, acid-base disorders and serious coagulation disturbance. Our group has been involved in the study of several aspects of the mechanism of action of scorpion toxins injected in animals. However some aspects of the envenomation, such as, the regional blood flow distribution to several organs need further studies. The aim of the present work was to determine the flow to different organs after intravenous injection of the gamma fraction of toxins isolated from the *T. serrulatus* scorpion venom adding more information that would contribute to a better understanding of the pathophysiology of scorpion sting envenomation. The fluorescent microspheres deposition technique was used to evaluate the regional blood flow and regional vascular resistance of several organs in anesthetized rats after *Tityus serrulatus* scorpion venom (Tx) injection. Fluorescent microspheres of different colors were injected into the left ventricle through the carotid artery. Reference blood samples were collected from the left femoral artery at baseline (before) and 5, 15 and 30 min after toxin injection. Tissue samples from the adrenal gland, spleen, brain, heart, liver, ileum, mesentery, muscle, skin, lung and kidney were harvested and prepared for fluorescent measurements using a spectrophotometer. Hemodynamic parameters were monitored continuously. Blood flow variation was detected in the tissue samples and were different among the organs. The most common finding was a decrease in flow, varying with time and the severity of the intoxication. However, intrinsic mechanisms of auto-regulation of blood flow and vascular resistance possibly protect the heart and the brain from hypoperfusion after Tx injection.

Keywords: Scorpion venom; Regional blood flow; Fluorescent microspheres; Rats; Circulatory shock; Respiratory dysfunction

Introduction

Scorpion envenomation is a relatively common event in tropical and subtropical countries, particularly in Brazil, where this condition is considered a public health problem [1]. In the state of Minas Gerais, Brazil, the envenomation caused by *Tityus serrulatus* scorpion species is responsible for high morbidity and mortality rates, especially in children and the elderly [2]. The northwest quarter of Belo Horizonte, the capital city of the state of Minas Gerais, reports one of the highest incidence rates, with an average of 10.37 envenomation events per 10,000 inhabitants [3]. The clinical manifestations of a sting start within a few minutes and include localised pain, hyperesthesia, hypersalivation, profuse sweating, vomiting, hyperexcitability, acute pancreatitis, gastric mucosa injury, hypertension followed by hypotension, respiratory and cardiovascular arrhythmias, cardiogenic shock, pulmonary edema, respiratory depression, and acid/base disorders [4,5].

Tityus serrulatus venom is a complex mixture of small neurotoxic proteins, interacting specifically with ionic channels in excitable membranes, and peptides which act on targets other than ion channels. The best studied proteins are relatively long chain toxins containing 60-70 amino acid residues mainly active on Na^+ channels. Short chain toxins, composed of 30-42 amino acid residues, form another big family of peptides that are mostly active on K^+ channels.

The effects of the crude venom as indicated by the clinical manifestations are similar to those seen after experimental envenomation with one of its toxic components, although the mechanism of action of toxins present in the venom may vary. The toxin used in the current experiments acts opening the sodium channels of autonomic nerve endings releasing chemical mediators which are responsible for the cardiovascular and respiratory disturbances. The severity of the envenomation depends upon the amount of venom injected by the scorpion sting, the age of the victim, or dose of venom used in experimental conditions [6].

The clinical manifestations are caused, in part, by neurotoxic peptides present in the Tx that are capable of stimulating the neuroendocrine and immunological axes by a mechanism that involves interaction with voltage-gated Na^+ channels of the autonomic system nerve endings. The activation of the Na^+ channels causes a massive release of catecholamines, acetylcholine, glucagon, cortisol, angiotensin-II, bradykinin and prostaglandins [6,7]. These agents induce the release of pro and anti-inflammatory cytokines such as

IL-1β, IL-1α, IL-6, IL-8, IL-10, NO, GM-CSF, TNF-α and INF-γ, which are involved in tissue injury and multiple organ failure [8,9].

The Tx also causes several systemic hemodynamic effects. An initial dose-dependent increase in the mean arterial pressure (MAP) provoked by catecholamine release from the adrenal glands and post-ganglionic nerve endings, is followed by hypotension, reduction of stroke volume (SV) and cardiac output (CO) and increase of systemic vascular resistance [10]. Spasms of the microvasculature related to catecholamine over-stimulation produce transitory myocardial ischemia and acute cardiac dysfunction [11,12]. Lung edema and respiratory arrhythmias have also been reported, as well as electrolyte and acid/base disorders [5,13,14]. After scorpion envenomation, decreased blood flow is observed in several organs including the kidneys [15], the gastrointestinal tract [16], and the spleen [17].

Blood flow distribution can be measured by the microsphere-deposition method first introduced by Rudolf and Heyman (1967) [18]. The basic principle is that the deposition of the markers is proportional to the tissue blood flow (per unit volume or mass of tissue). The original method utilized radiolabeled microspheres. However, the use of fluorescent microspheres has decreased both the health risks and the costs of the method. The accuracy of the fluorescent markers in the assessment of organ perfusion in small animal models has been validated by several investigators [19-21]. However, there are no previous reports in which the fluorescent microsphere technique has been used to assess organ perfusion following scorpion envenomation.

In the present study, we used the fluorescent microspheres method to investigate the effect of *T. serrulatus* scorpion toxin envenomation on regional organ blood flow in rats.

Materials and Methods

Scorpion toxin

The experiments were conducted using the purified toxic fraction T1 of the *Tityus serrulatus* scorpion crude venom. The extraction method involved a combination of water extraction and column chromatography on Sephadex G-25 [22].

Animals

Fifty-one male Wistar rats (Rattus norvegicus), weighing 283.1 ± 47.6 g, were allocated in groups according to the time of blood flow assessment before and after toxin injection (5, 15 and 30 min). They were fed with standard laboratory chow, provided with water ad libitum, and housed in appropriate animal cages in the animal facility of our Institution. All animals were cared for in accordance with ethical recommendations approved by the Ethics Committee for Animal Experimentation of the Universidade Federal de Minas Gerais, Brazil.

Surgical procedures

The animals were anesthetised with an intraperitoneal injection of urethane (1.4 g/kg), after which a tracheostomy was performed. The animals breathed spontaneously through a plastic tracheal cannula. The left jugular vein and right and left femoral arteries were cannulated with polyethylene (PE) tubing: PE50 heat-fused to PE10 (0.28 mm i.d.). A catheter was placed into the right carotid artery and advanced to the left ventricle for the infusion of different color fluorescent-labelled polystyrene microspheres with nominal diameter of 15 μm. Tx (250 μg/kg) was injected into the left jugular vein. The right femoral artery catheter was connected to a pressure transducer (MP150 Data Acquisition System, BIOPAC System Inc, Goleta, CA, USA) for continuous monitoring of the mean arterial pressure (MAP) and the heart rate (HR). The left femoral artery catheter was coupled to an infusion pump (Minipuls 3, Gilson, Villiers Le Bel, France) calibrated at 0.85 ml/min for blood sample collections as needed. Fluorescent polystyrene microspheres (FluorSpheres®, Invitrogen Molecular Probe®, Eugene, OR), 15 μm in diameter, were suspended in solution (0.15 M of NaCl 0.05%, Tween 20, and 0.002% Thimerisol). Microspheres containing red fluorescent dyes (absorption/emission wavelength 580/605 nm), blue-green (505/515 nm), blue (625/645 nm), and orange (540/560 nm) were used. To ensure uniform dispersion of the microspheres in the blood, by minimizing aggregation, microsphere suspension was ultrasonicated and vigorously vortexed for one minute prior to injection. After sonication, 0.3 ml of the microsphere solution, approximately 300,000 microspheres, was aspirated into a 1 ml syringe (Becton Dickinson Ind. Cir. Ltda., Curitiba, PR, Brazil). Withdrawal of blood started 10 s before injection of the microspheres and was continued until the total time of 1, 5 min. The carotid artery catheter was connected to the 1 ml syringe containing the microsphere solution of a chosen color. The catheter was flushed with 2 ml of RL during the last 60 seconds of blood removal to prevent microspheres adhesion to the inner surface of the catheter and to replace the volume of blood removed. The right femoral artery catheter was connected to a peristaltic roller pump (Minipuls 3 Gilson, Villiers Le Bel, France) preset to remove blood at a rate of 0.7 ml/min into a test tube. The first reference blood sample was obtained at the beginning of hemodynamic recording to assess the baseline blood flow and vascular resistance, and the second reference blood sample was collected 5, 15 or 30 min after Tx injection. The animals were exsanguinated immediately after the second blood collection, and the following organs and tissues were harvested: both adrenal glands, spleen, brain, heart, liver, terminal ileum, mesentery, right rectus abdominal muscle (1.5 cm^2), abdominal skin (1.5 cm^2), left lung and both kidneys.

Tissue sample preparation

All organs, tissues and blood samples were weighed and placed into separate centrifuge tubes (35 mm × 105 mm) (Sorvall Legend Mach 1.6-R, Thermo Scientific, Waltham, MA) containing 8 ml of a lysing solution (4 M KOH+2% Tween 80 in ethanol, total final solution=200 ml). Samples were kept at 50°C overnight and centrifuged at 10000 rpm for 20 min (Sorvall Legend Mach 1.6R Centrifuge, ThermoFisher Scientific, Waltham, MA, USA). The supernatant was discarded, and 8 ml of 2% Triton-X, 8 ml of deionised water and 4 ml of 98% ethyl acetate (Labsynth, Diadema, SP, Brazil) were added to the pellet. The tubes were vortexed and sonicated to facilitate homogenisation. The final solution was kept in a dark room for 10 min to avoid photobleaching of the fluorescent dyes, and readings were performed within one hour before the fluorescence assessment.

Determination of fluorescence

All samples were loaded into a quartz cuvette and the fluorescence was assessed with a spectrophotometer (Shimadzu Scientific Instruments UV-3600-UV-VIS-NIR, Columbia, MD) with an excitation/emission wavelength predetermined according to the color

of fluorescent-labelled microspheres used. Fluorescence measurements were made with excitation and emission slit widths of 3 nm.

Calculation of organ blood flow

The deposition of microspheres in an organ is proportional to the fluorescence intensity. The blood flow to each organ was calculated according to previously published methods [19,21,23,24]. The number of microspheres (MS) in a particular sample was calculated by multiplying the actual fluorescence of the sample (FS) by the conversion factor (CF) obtained for the fluorescent dye used in the sample (MS=FS × CF). The CF is the arithmetic mean of the values for fluorescence of a 98% ethyl acetate solution containing, respectively 2500, 1250 or 625 microspheres/ml. The blood flow to an individual organ (Q) was calculated using the number of microspheres in the sample (MS), the number of microspheres in the reference blood sample (MRBS), the weight of the sample (W), and the reference flow (RF), as in the formula: Q = MS/MRBS × RF/W. To obtain the reference flow (RF), the density of blood (1.06 ml/g) was multiplied by the blood sample withdrawal speed (1.5 ml/min), and then divided by the weight of the reference blood sample. Cardiac output (CO, ml/min) was calculated by taking the amount of microspheres injected in the left ventricle (MLV=300,000) divided by the number of microspheres in the reference blood sample (MRBS) multiplied by the reference flow (RF), as in the formula: CO=MLV/MRBS × RF. Cardiac output was expressed as ml/min or as ml/min/100g of body weight. Cardiac index (CI) was calculated using the formula: CI=CO/body surface area (in ml/min/cm^2) [25]. The systolic volume (SV) was calculated by cardiac output (CO) divided by HR, as in the following formula: SV=CO/HR.

Statistical analysis

The results were expressed as mean ± SEM. The statistical analysis was performed using ANOVA with Tukey´s post-hoc test. The criterion for significance was set at $P<0.05$.

Results

Hemodynamic response

Decreased SV and CO were first observed five minutes after intoxication and persisted throughout the experiment. A significant reduction in HR was noted 15 min after Tx injection.

Total peripheral vascular resistance increased continuously between 15 min and 30 min, consistent with the CO reduction. The MAP increased immediately after Tx injection and remained elevated throughout the experiment. The general hemodynamic effects of Tx are shown in Table 1.

Hemodynamic Parameters		Time after Tx (min)		
	Baseline	**5**	**15**	**30**
Cardiac output (ml/min)	266.2 ± 13.6	150.4 ± 22.8	200.6 ± 8.5	189.3 ± 9.0
Cardiac output (ml/min/100 g)	101.9 ± 2.80	54.6 ± 11.79[a]	71.2 ± 5.85[a]	62.35 ± 9.65
Cardiac index (mil/min/SA)	0.64 ± 0.00	0.35 ± 0.08	0.47 ± 0.02	0.42 ± 0.02
Systolic Volume (ml/stroke)	0.66 ± 0.02	0.38 ± 0.05[a]	0.56 ± 0.06	0.48 ± 0.08[a]
Total Peripheral Resistance (mmHg.ml/g.min)	0.96 ± 0.04	0.61 ± 0.17	1.58 ± 0.09[ab]	1.65 ± 0.43[ab]
Mean Arterial Pressure (mmHg)	87.97 ± 1.45	101 ± 4.15[a]	110.6 ± 2.54[a]	128 ± 4.77[abc]
Cardiac Frequency (bpm)	402 ± 5.26	394.7 ± 3.67	357.3 ± 11.16[a]	394 ± 12.08

The regional blood flow was determined by the fluorescent microspheres method.
Values are expressed as the mean ± SEM. Tx-5, Tx-15, and Tx-30 indicate groups of animals 5, 15 and 30 minutes, respectively, after injection of fraction T1 of scorpion venom (250 µg/Kg).
Number of animals/group: Baseline n ≥ 7; Tx-5 n ≥ 6; Tx-15 n ≥ 6; Tx-30 n ≥ 6.
(* The "n" of each group varied according to the organ evaluated, due to sample loss and/or exclusion of outlier values after statistical analysis)
SA-Body surface area in cm^2
[a]= different from baseline; [b]= different from Tx-5; [c]= different from Tx-15. Differences were considered significant when $P<0.05$.

Table 1: Effect of *T. serrulatus* scorpion toxin (fraction T1) on rat hemodynamic parameters.

Regional blood flow and vascular resistance (VR)

Tx injection did not cause statistically significant changes in the blood flow to adrenal glands or ileum when compared to baseline values (time zero). Similarly, Tx did not significantly affect the perfusion of the heart, despite a significant increase in the regional vascular resistance at 30 min time points. Brain and mesenteric blood flows were significantly reduced at 15 min, but returned to near baseline values at 30 min. A significant increase in the regional vascular resistance at 15 and 30 min time points was noted in brain and mesentery, respectively. Liver and skin showed increases in blood flow at 30 min after toxin injection. The regional vascular resistance was significantly reduced in the skin at 15 min time point. The blood flow to spleen, kidneys and lungs was significantly reduced along the whole time beginning just at 5 min after Tx injection. Alteration caused by scorpion toxin from *Tityus serrulatus* venom depends on the organ considered. Summary of data of blood flow to different organs is depicted in Figure 1.

Tables 2 and 3 depict data for regional blood flow and vascular resistance, respectively.

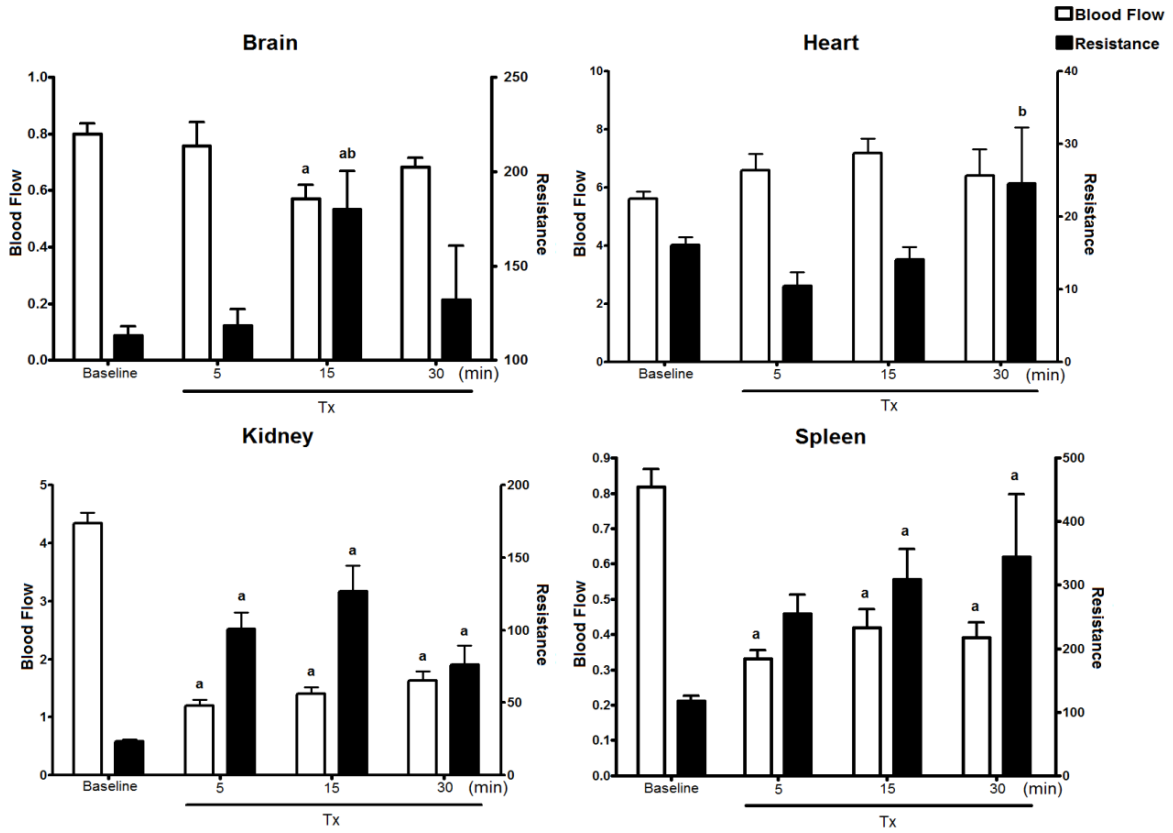

Figure 1: Regional blood flow (ml.min^{-1}.g^{-1}) and regional vascular resistance (mmHg.ml^{-1}.min.g) in brain, heart, kidney and spleen after scorpion venom injection in rats. Regional blood flow was determined according to the fluorescence microsphere method. Columns represents the means and bars the SEM. Note that Tx caused a decrease in blood flow to kidney and spleen, but did not significantly alter blood flow to brain and heart. Baseline indicates the blood flow before Tx injection. Tx-5, Tx-15, and Tx-30 indicate groups of animals 5, 15 and 30 min, respectively, after injection of fraction T1 (250 μg/Kg) of scorpion venom. [a]=different from baseline; [b]=different from Tx-5. Differences were considered significant when P<0.05.

Organs		Time after Tx (min)		
	Baseline	**5**	**15**	**30**
Adrenal	4.05 ± 0.31	4.37 ± 0.46	5.56 ± 0.69	3.88 ± 0.45
Brain	0.79 ± 0.03	0.75 ± 0.08	0.57 ± 0.04[a]	0.68 ± 0.03
Heart	5.59 ± 0.25	6.58 ± 0.57	7.18 ± 0.48	6.39 ± 0.91
Ileum	0.61 ± 0.04	0.67 ± 0.09	0.63 ± 0.05	0.82 ± 0.04
L. Kidney	3.97 ± 0.17	1.2 ± 0.18[a]	1.37 ± 0.20[a]	1.55 ± 0.14[a]
R. Kidney	4.69 ± 0.27	1.18 ± 0.15[a]	1.43 ± 0.09[a]	1.73 ± 0.29[a]
Liver	0.39 ± 0.02	0.53 ± 0.07	0.40 ± 0.03	0.65 ± 0.10[ac]
Lung	1.38 ± 0.16	0.36 ± 0.07[a]	1.25 ± 0.21[b]	1.46 ± 0.11[b]
Mesentery	0.66 ± 0.04	0.45 ± 0.08	0.32 ± 0.03[a]	0.65 ± 0.11[c]
Muscle	0.15 ± 0.01	0.14 ± 0.02	0.18 ± 0.03	0.17 ± 0.02

Skin	0.07 ± 0.01	0.17 ± 0.04	0.10 ± 0.01	0.25 ± 0.06[ac]
Spleen	0.81 ± 0.04	0.33 ± 0.02[a]	0.41 ± 0.05[a]	0.39 ± 0.04[a]

The regional blood flow was determined by the fluorescent microspheres method.
Values are expressed as the mean ± SEM. Tx-5, Tx-15, and Tx-30 indicate groups of animals 5, 15 and 30 minutes, respectively, after injection of fraction T1 of scorpion venom (250 µg/Kg).
Number of animals/group: Baseline n ≥ 7; Tx-5 n ≥ 6; Tx-15 n ≥ 6; Tx-30 n ≥ 6.
(* The "n" of each group varied according to the organ evaluated, due to sample loss and/or exclusion of outlier values after statistical analysis.)
[a]=different from baseline; [b]=different from Tx-5; [c]=different from Tx-15. Differences were considered significant when P<0.05.

Table 2: Regional blood flow ($ml.min^{-1}.g^{-1}$) to different organs after injection of T1 fraction of *T. serrulatus* scorpion venom in rats.

Organs	Time after Tx (min)			
	Baseline	**5**	**15**	**30**
Adrenal	25.72 ± 1.91	20.25 ± 2.06	19.37 ± 2.37	22.16 ± 6.02
Brain	113 ± 5.18	118.2 ± 8.97	179.9 ± 20.28[ab]	132 ± 28.72
Heart	16.09 ± 1.03	10.39 ± 1.90	14.06 ± 1.75	24.53 ± 7.68[b]
Ileus	145.4 ± 7.53	135.8 ± 19.26	157.9 ± 19.84	143.7 ± 25.51
L. Kidney	23.96 ± 1.87	103.4 ± 17.50[a]	120.4 ± 24.72[a]	72.47 ± 17.02[a]
R. Kidney	22.59 ± 1.45	97.99 ± 14.13[a]	131.9 ± 27.25[a]	79.65 ± 21.87[a]
Liver	198.3 ± 13.18	215 ± 31.77	213.8 ± 27.36	159.3 ± 50.81
Lung	105.5 ± 17.24	102.7 ± 39.88	208.4 ± 30.75[a]	95.42 ± 21.15
Mesentery	241.1 ± 15.14	299.9 ± 54.74	348.4 ± 43.6	585.2 ± 220.4[a]
Muscle	501.5 ± 48.68	683 ± 130.2	289.7 ± 39.90	833.8 ± 228.3[c]
Skin	998.4 ± 35.58	1032 ± 204.9	444.4 ± 96.45[ab]	875.7 ± 198.6
Spleen	117.4 ± 7.80	254.2 ± 30.38	308.3 ± 48.35[a]	344.2 ± 98.64[a]

The regional blood flow was determined by the fluorescent microspheres method.
Values are expressed as the mean ± SEM. Tx-5, Tx-15, and Tx-30 indicate groups of animals 5, 15 and 30 minutes, respectively, after injection of fraction T1 of scorpion venom (250 µg/Kg). Number of animals/group: Baseline n ≥ 11; Tx-5 n ≥ 6; Tx-15 n ≥ 6; Tx-30 n ≥ 6. (* The "n" of each group varied according to the organ evaluated, due to samples loss and/or exclusion of outlier values after statistical analysis). [a]=different from baseline; [b]=different from Tx-5; [c]=different from Tx-15. Differences were considered significant when P<0.05.

Table 3: Effect of T1 fraction of *T. serrulatus* scorpion toxin on regional vascular resistance ($mmHg.ml^{-1}.min.g$) in rats.

Discussion

The microsphere deposition method provides more detailed information regarding regional blood flow than flow probes or techniques based on molecular tracer washout. One of the advantages of the microsphere deposition methodology is that it does not alter toxin-induced effects on blood flow or hemodynamic parameters [20,21].

The reliability of the method is usually confirmed by similar results in organ perfusion between the two kidneys as in the present study [26].

The used dose of T1 fraction of Tx (250 µg/kg) was chosen based on animal survival curves, clinical manifestations, and hemodynamic parameters. This is the minimal dose necessary to promote clear-cut manifestations of moderate envenomation, including respiratory depression, pulmonary edema, arterial hypertension and hypersalivation, without causing severe shock and death of the animal. Higher doses would certainly lead to severe shock and death of the animals.

Experiments were performed on urethane anesthetized rats. This drug is widely used nowadays for animal experimentation because of its long duration of action, skeletal muscle relaxant properties and the short time required to attain a surgical level of anesthesia [27]. It is known that urethane inhibits cardiovascular responses mediated by sympathoadrenal stimulation [28]. However, urethane is considered one of the most appropriate anesthetics because its action on the cardiac function is relatively mild when used at relatively low doses [29]. In addition, the level of anesthesia and the cardiovascular parameters remained stable for the whole duration of the experiments with no need for additional doses, a desirable condition for this kind of experiment. The potential interference of urethane with the blood flow to some organs was minimized in our experiments as we only have determined the baseline blood flow after hemodynamic parameters stabilization. In this way the alterations observed after Tx-injection

were probably due to the toxin effect itself and not to urethane anesthesia. The urethane- raised objections used by some investigators [30-32] might be due, at least in part, to different doses, different routes of administration (subcutaneous, intravenous, intraarterial and intraperitoneal), as well as to combinations of anesthetics.

Crude scorpion venom naturally injected by the scorpionor in experimental conditions acts on post-ganglionic nerve endings of the autonomic nervous system releasing catecholaminergic and cholinergic mediators, among other substances, that can lead to shock of uncertain or mixed etiology with a hypovolemic-like pattern, even in the absence of active bleeding focus or external fluid losses with consequent pronounced impact on organs involved in the immune, cardiovascular, pulmonary systems, and energy metabolism. The responses to the crude venom are quite similar to those seen after injection of one of its isolated toxic fractions as in the present study [33].

The dose of Tx used in the study induced an increase in MAP within the first 5 min after injection. However, total peripheral vascular resistance did not increase significantly until the 15 min time point, suggesting that this parameter involves a more complex response, involving the release of adrenergic and cholinergic neurotransmitters in blood stream, which takes longer to occur after the toxin dose injected. However, the cardiac output and cardiac index decreased significantly within the first 5 min [10].

Other regulatory substances such as glucagon, cortisol, angiotensin II, and insulin may also be altered by Tx injection and may play a role in the Tx-induced hemodynamic response. The Tx effects could lead to energetic deficit syndrome, cardiovascular disturbances, and peripheral circulatory failure [34]. A role for glucagon in hemodynamic effects of Tx injection is supported by the fact that insulin infusion reverses scorpion toxin-induced hemodynamic changes and pulmonary edema [35].

Our study showed a reduction in regional blood flow to the majority of the organs after Tx injection. However, the effects on blood flow reduction varied in magnitude and time of occurrence and were frequently associated with increased vascular resistance. The scorpion venom excites the sympathetic postganglionic nerve endings inducing release into the blood of both norepinephrine and epinephrine, with slightly different effects in exciting the alpha and beta receptors. Therefore, the relative effects of norepinephrine and epinephrine on different effector organs are determined by the types of receptors in those organs. These variations are likely to be related to the physiologic capability of the microvasculature to control the blood flow in proportion to metabolic needs, and could explain the variability of blood flow depending on the distribution of adrenergic and cholinergic receptors of each organ. The sympathetic stimulation does not cause significant constriction of either the cerebral or the cardiac vessels. In addition, in both vascular beds, local blood flow autoregulation is excellent, which prevents moderate decreases in arterial pressure from significantly decreasing their blood flows. Therefore, blood flow through the heart and brain is maintained essentially at normal levels as long as the arterial pressure does not fall below about 70 mm Hg, despite the fact that blood flow in some other areas of the body might be decreased to as little as one third to one quarter normal by this time because of vasoconstriction .

Regional blood flow to the heart did not decrease after toxin injection, despite a significant increase in regional vascular resistance. We speculate that protective autoregulatory mechanisms of the myocardium prevented a decrease in blood flow. An analogous but less effective mechanism most likely prevented a decrease in regional blood flow to the brain, at both 15 and 30 min time points after Tx injection. Two views, the metabolic theory and the myogenic theory have been proposed to explain this acute autoregulation mechanism. In accordance with the metabolic theory blood flow autoregulation occurs when the arterial pressure becomes too great, the excess flow provides too much oxygen and too many other nutrients to the tissues and "washes out" the vasodilators released by the tissues. These nutrients (especially oxygen) and decreased tissue levels of vasodilators then cause the blood vessels to constrict and return flow to nearly normal despite the increased pressure. The myogenic theory explains the phenomenon of autoregulation based on the observation that sudden stretch of small blood vessels causes the smooth muscle of the vessel wall to contract reducing the blood flow nearly back to normal. The myogenic response is inherent to vascular smooth muscle and can occur in the absence of neural or hormonal influences [36].

Although our results did not show a decrease in the blood flow to the heart, several studies have demonstrated that the inotropic effect of scorpion toxin induces a large increase in myocardial oxygen consumption. The increased oxygen consumption can lead to dose-dependent myocardial hypoxia, impairment of myocardial function and arrhythmias [10-12].

The mechanisms involved in local blood flow regulation act independently of systemic stimuli such as circulating hormones and the autonomic nervous system. These mechanisms can be demonstrated experimentally in isolated perfused organs. Under these conditions, the balance between local regulation and extrinsic factors will ultimately determine local blood inflow [37].

In the present study, the correlation between organ perfusion and vascular resistance was more strikingly demonstrated in the spleen and kidneys. We postulate that in these organs, toxin-induced catecholamine release caused vasoconstriction followed by tissue hypoperfusion. Consistent with this hypothesis, increased renal release of intrinsic vasoconstrictor substances (e.g. arachidonic acid, thromboxane and leukotriene metabolites) have been shown to contribute to reduced renal blood flow, which in turn causes metabolic acidosis and hyperkalemia [5].

The initial reduction in pulmonary blood flow 5 min after Tx injection was followed by an increase to near-baseline levels at the 30 min time point. This observation can be partially explained by the hyperdynamic cardiovascular status of the animals and the subsequent reperfusion injury. A previous study on isolated rabbit lungs demonstrated that a transient increase in pulmonary artery to near-baseline levels at the 30 min time point. A previous study on isolated rabbit lungs demonstrated that a transient increase in pulmonary artery pressure was caused by humoral non-catecholaminergic factors released by scorpion toxin. These factors were capable of provoking lung edema [38]). Furthermore, platelet activating factor, released as part of the inflammatory response to Tx injection, provokes vasoconstriction and increases pulmonary vascular resistance, with the subsequent development of reperfusion injury [39-41].

We also speculate that the dual blood supply of the liver may have protected it from hypoperfusion after Tx injection. Our results showed a significant increase in blood flow to the liver at the 30 min time point without significant changes in regional vascular resistance. However, because the fluorescent microsphere technique assesses only the arterial blood flow, our results might have underestimated liver

perfusion originating from the portal vein [42-44]. Studies have shown that both increase in carbon dioxide as well as in hydrogen ion concentration cause vasodilation. Furthermore, adenosine accumulates in the liver during ischemic events. This nucleoside causes arterial vasodilatation and increases local blood flow, thus providing another protective mechanism. Adenosine is considered by many physiologists to be the most important vasodilator involved in local blood flow control, and experimental studies indicate that it might have a significant role in the regulation of the buffer response. In addition it can be postulated that NO may play a role in the control of the liver blood flow promoting vasodilation. All these events might explain the hepatic blood flow increase following scorpion venom injection [36].

One unexpected finding was that blood flow to the skin increased significantly at 30 min. This observation could be partially explained by the decrease in regional vascular resistance at the 15 min time point. However, according to a previous study, blood flow to a non-vital organ such as the skin should have decreased after Tx injection [45]. It should be noted that the presence of arteriovenous anastomoses allows for large variations in the blood flow to the skin: from barely detectable to as much as 30% of the total cardiac output [44]).

We are aware of the limitations of this study. First, extrapolation of the results of the experimental fluorescent microsphere technique to a clinical scenario will require further validation. Second, we did not perform a dose-response analysis of the Tx envenomation, thus limiting the analysis of the physiologic responses to a single dose. This is justified by the acquired experience in this field through several papers already published by the same group on the mechanism of action of scorpion toxin. Third, the period of observation after Tx injection was only 30 min. It might well be possible that the blood flow, could show different parameters with consequent modification of tissue perfusion at later times [5,46]. However the dose of Tx used leads to a high mortality rate should the experiment be prolonged. Finally, the intrinsic and extrinsic autoregulatory mechanisms involved in local blood flow control after the scorpion toxin injections were not investigated in this study. All these considerations point out to a need to perform further experiments in the future to elucidate the complex regulatory mechanisms occurring after different kind of insults, leading to hemodynamic shock.

Conclusion

Intravenous injection of scorpion toxin induces significant changes in regional blood flow to several organs. A reduction in blood flow was most frequently noted and was generally associated with an increase in regional vascular resistance which varies among different organs studied. The blood flow to brain and heart was less affected by T1 fraction of *T. serrulatus* scorpion venom.

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