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Effects of *Tityus serrulatus* scorpion venom on lung mechanics and inflammation in mice

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ABSTRACT

The present study evaluated the effects of an intramuscular injection of *Tityus serrulatus* venom (TsV) (0.67 μg/g) on lung mechanics and lung inflammation at 15, 30, 60 and 180 min after inoculation. TsV inoculation resulted in increased lung elastance when compared with the control group ($p < 0.001$); these values were significantly higher at 60 min than at 15 and 180 min ($p < 0.05$). Resistive pressure (ΔP_1) values decreased significantly at 30, 60 and 180 min after TsV injection ($p < 0.001$). TsV inoculation resulted in increased lung inflammation, characterised by an increased density of mononuclear cells at 15, 30, 60 and 180 min after TsV injection when compared with the control group ($p < 0.001$). TsV inoculation also resulted in an increased pulmonary density of polymorphonuclear cells at 15, 30 and 60 min following injection when compared to the control group ($p < 0.001$). In conclusion, *T. serrulatus* venom leads to acute lung injury, characterised by altered lung mechanics and increased pulmonary inflammation.

1. Introduction

Tityus serrulatus is one of most venomous scorpions. Its venom is made up of water-soluble and water-insoluble proteins, among which tityustoxin is the most toxic component (Nunan et al., 2004). The vast majority of toxins in *T. serrulatus* venom have neurotoxic effects (Nunan et al., 2004; Vasconcelos et al., 2004), acting through ion channels and the release of neurotransmitters (Vasconcelos et al., 2004). Scorpion bites are highly relevant, as their effects can lead to severe and even fatal situations (Guidine et al., 2008). *T. serrulatus* is considered as the most dangerous scorpion in South America, with about 10,000 cases both envenomation reported each year in Brazil alone (Brazilian National Health Foundation, 1998). A large number of complications follow scorpion envenomation, such as hyperglycaemia and suppression of insulin secretion (Murthy and Haghnazari, 1999), counter-regulatory hormones (Corrêa et al., 1997; Murthy and Haghnazari, 1999), respiratory failure (Sofer and Gueron, 1988), lung oedema (Amaral et al., 1993; Mesquita et al., 2002),

Abbreviations: TsV, *Tityus serrulatus* venom.

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arrhythmia (Ismail and Abd-Elsalam, 1988) and convulsion (Ismail et al., 1990). There are no studies that evaluate the specific effects of *T. serrulatus* venom on lung mechanics in mice (Andrade et al., 2004). Therefore, the time-dependent effects of *T. serrulatus* venom on lung mechanics and inflammation (as performed in the present study) have never before been evaluated.

The mechanical properties of the respiratory system, such as elastic, resistive and viscoelastic components, play a central role in the diagnosis of pulmonary response to different situations, such as asthma (Xisto et al., 2005), chronic obstructive pulmonary disease (Mall et al., 2008), acute respiratory distress syndrome (Santos et al., 2006) and acute lung injury (Santos et al., 2006). Alterations in respiratory system components lead to altered lung mechanics, characterised by histological abnormalities, oedema, haemorrhaging, inflammation and increased deposition of matrix extra-cellular proteins (Santos et al., 2006). Evaluating the effects of TsV on lung mechanics and inflammatory response could be helpful in understanding the harmful effects of TsV.

The aim of the present study was to evaluate the mechanical properties and perivascular lung inflammation resulting from the intramuscular administration of TsV. For this purpose, elastic, resistive and viscoelastic mechanical changes in the lungs as well as lung perivascular mononuclear and polymorphonuclear cells infiltration were determined at 15, 30, 60 and 180 min following an intramuscular injection of TsV in mice.

2. Material and methods

2.1. Venom and reagent

Dried venom was donated by the Butantan Institute and all reagents used here had a high degree of purity (ACS or HPLC grade).

2.2. Venom solution

The venom (mg) was dissolved in a sterile saline solution until complete solubilisation. The resting material was centrifuged at 4500*g* for 5 min. The resulting supernatant was recovered and reserved for the biological assays.

2.3. Animals and experimental groups

Male Swiss mice (18–20 g) were kept housed in temperature-controlled conditions, with relative humidity of 50–60%, 12 h/12 h dark cycle/light cycle and received water and food *ad libitum*. The study protocol was approved by Ethics Committee for animal experimentation of University of Vale do Paraíba (process number L059/2005/CEP).

Thirty mice were divided into five groups ($n = 6$): control group (CG); 15 min after TsV inoculation (G15); 30 min after TsV inoculation (G30); 60 min after TsV inoculation (G60); and 180 min after TsV inoculation (G180).

2.4. Lethal dose (LD_{50})

The mean lethal dose from the intramuscular injection of TsV in mice is 0.6 μg/g body weight (BW). This LD_{50} was previously determined in our laboratory through the intramuscular injection of five different doses (0.67; 0.83; 1.00; 1.17 and 1.33 μg/g BW) in five groups of mice each and recording the occurrence of death within a 24-h period.

2.5. Experimental procedure

TsV (0.67 μg/g) was dissolved in 50 μL of sterile saline solution and administered through an intramuscular injection. Control animals received 50 μL of sterile saline solution. At 15, 30, 60 and 180 min following the venom injection, the animals were sedated [pentobarbital sodium (0.7 μL)] and a snug-fitting cannula (1.1 mm ID) was introduced into the trachea and then connected to a computer-controlled mechanical ventilator (Samay MVR16xp, Montevideo, Uruguay).

The anterior chest wall was surgically removed. Mechanical ventilation [frequency of 100 breaths min^{-1}, tidal volume of 0.2 ml and adequate amount of positive end-expiratory pressure (PEEP = 2 cmH_2O)] was administered immediately prior to entering the pleural cavity. The PEEP level was determined as follows: before the pleural cavity was opened, the ventilator was disconnected at end expiration and the airways were occluded. Following the pleural incision, there was an increase in transpulmonary pressure (PL), which corresponded to the elastic recoil pressure of the lung at relaxation volume. Thereafter, the same pressure was applied to the lungs as PEEP (Silveira et al., 2004).

A pneumotachograph (Mortoloa and Naworaj, 1983) was connected to the tracheal cannula for the measurement of airflow (V') and changes in lung volume (V_T). The flow resistance of the equipment (R_{eq}) (including the tracheal cannula) was constant up to flow rates of 26 ml/s and amounted to 0.12 $cmH_2O\ ml^{-1}\ s$. Resistive pressure ($R_{eq}.V'$) of the equipment was subtracted from pulmonary resistive pressure so that the results would represent intrinsic values. As there were no abrupt changes in diameter in the circuit, errors were avoided in flow resistance measurements (Chang and Mortola, 1981). Flow and pressure signals were measured by transducers connected to the pneumotachograph and then to an electromyograph signal conditioner (EMG system, Brazil) with 8 channels of analogical input, 1000× amplification, sampled at 250 Hz, with a 12-bit analogue-to-digital converter and stored on a microcomputer. All data were collected using software Windaq™ 2.81 (DATAQ Instruments, Akron, Ohio, USA) (Nonaka et al., 2008).

2.6. Measurement of pulmonary mechanics

Pulmonary mechanics were measured from end-inspiratory occlusions after constant flow inflations (Bates et al., 1985; Silveira et al., 2004; Nonaka et al., 2008). In an open-chest preparation, tracheal pressure reflects transpulmonary pressure (PL). After end-inspiratory occlusion, there is an initial rapid drop in PL (ΔP_1) from the pre-occlusion value down to an inflection point (P_i), followed by a slow pressure decay (ΔP_2) until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the lung (P_{el}). ΔP_1 selectively reflects pressure dissipated

against pulmonary resistance in normal animals and humans and ΔP_2 reflects viscoelastic properties (stress relaxation) and/or homogeneities of lung tissues, together with a miniscule contribution from the pendelluft in normal situations (Saldiva et al., 1992; Silveira et al., 2004; Nonaka et al., 2008). Total pressure drop (ΔP_{tot}) is equal to the sum of ΔP_1 and ΔP_2. Lung static elastance (E_{st}) was calculated by dividing P_{el} by V_T. Dynamic elastance of the lung (E_{dyn}) was determined by dividing by V_T. ΔE was calculated as the $E_{dyn} - E_{st}$ difference. The measurement of pulmonary mechanics was performed 10–15 times in each animal. The experiments did not last more than 40 min. Data analysis was performed using the ANADAT software program (RHT InfoData, Montreal, Canada).

2.7. Lung histology

Histopathological analysis was performed on the excised lungs of all animals. Immediately following the measurements of the mechanics, the animals were exsanguinated through the abdominal aorta and vena cava. The chest wall was opened and 0.3 ml of formalin was instilled into the lungs. The lungs were surgically removed *en bloc* and fixed in formalin for 48 h. Slices (5 μm in thickness) were stained with haematoxylin–eosin and examined by a pathologist without any knowledge as to the treatment group. Morphometric analysis of perivascular lung inflammation (five peribronchiolar arteries per animal) was performed through a differential cell count (mononuclear and polymorphonuclear), using a point count method (Vieira et al., 2007a,b, 2008; Nonaka et al., 2008). Using a 100-point grid with a known area (62,500 μm^2 at 400× magnification) attached to the eyepiece of the microscope, the number of points occurring between the perivascular areas (located between the external limit of arterial smooth muscle layer and the artery adventitia) was computed. The perivascular area in each field was calculated according to the number of points hitting in the perivascular area as a proportion of the total grid area. The number of mononuclear and polymorphonuclear cells was counted in this area. For each cell type, cell density was determined as the number of mononuclear and polymorphonuclear cells in each field divided by tissue area. Measurements were expressed as cells/μm^2. The results were then transformed into cells/mm^2 by adjusting the units (Vieira et al., 2007a,b, 2008; Nonaka et al., 2008).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls post-hoc test (parametric data) and one-way analysis of variance on Ranks (ANOVA on Ranks) followed by Dunn's post-hoc test (non-parametric data) were used for comparisons of the different parameters between groups. Values were expressed as mean ± SD for parametric data and as median (variance) for non-parametric data. The level of significance was set at $p < 0.05$.

3. Results

3.1. Pulmonary mechanics

For evaluation of pulmonary mechanics, were taken the data of airway flow variance, volume and PEEP. The flow (1.33 ml/s), volume (0.2 ml) and PEEP (2 cmH_2O) were constant during all data collection (Table 1).

3.2. Elastic lung properties

Fig. 1A and B respectively shows that TsV increased static elastance at 15, 30, 60 and 180 min after injection when compared with the control group ($p < 0.001$), with a statistically greater increase in G60 when compared with the G15 group ($p < 0.05$). Dynamic elastance also exhibited increased values at 15, 30, 60 and 180 min after TsV injection when compared with the control group ($p < 0.001$) and in G60 when compared with the G15 group ($p < 0.05$). Peak static and dynamic elastance values were found 60 min after TsV venom injection. Fig. 1C demonstrates that there were no differences in ΔE values [variation in pulmonary elastance (difference between dynamic and static elastance)] between the experimental groups ($p > 0.05$).

3.3. Resistive and viscoelastic lung properties

Fig. 2A shows that TsV decreased ΔP_1 values, which represent the resistive properties of pulmonary tissue, at 30, 60 and 180 min after injection when compared with the control group ($p < 0.001$). Fig. 2B shows there were no changes in ΔP_2 values, which represent the viscoelastic properties of pulmonary tissues ($p > 0.05$). Fig. 2C shows that TsV injection decreased ΔP_{tot} values, which represent the total lung pressure variation, at 30 and 60 min after injection when compared with the control group ($p < 0.001$).

3.4. Lung histology

Fig. 3A shows that TsV resulted in increased perivascular infiltration of mononuclear cells at 15, 30, 60 and 180 min after injection when compared with the control group ($p < 0.001$). Fig. 3B shows that TsV resulted in increased perivascular infiltration of polymorphonuclear cells at 15, 30 and 60 min after injection when compared with the control group ($p < 0.001$), returning to control values at 180 min.

A qualitative analysis of lung tissue was also performed, which revealed that TsV injection resulted in a slight

Table 1
Parameters for pulmonary mechanics evaluation.

$n = 30$	Flow (ml/s)	Volume (ml)	PEEP (cmH_2O)
Control	1.33 ± 0.00	0.20 ± 0.00	2.00 ± 0.00
G15	1.33 ± 0.00	0.20 ± 0.00	2.00 ± 0.00
G30	1.33 ± 0.00	0.20 ± 0.00	2.00 ± 0.00
G60	1.33 ± 0.00	0.20 ± 0.00	2.00 ± 0.00
G180	1.33 ± 0.00	0.20 ± 0.00	2.00 ± 0.00

Control, injected with saline; G15, 15 min after TsV injection; G30, 30 min after TsV injection; G60, 60 min after TsV injection; G180, 180 min after TsV injection; PEEP = positive end-expiratory pressure.

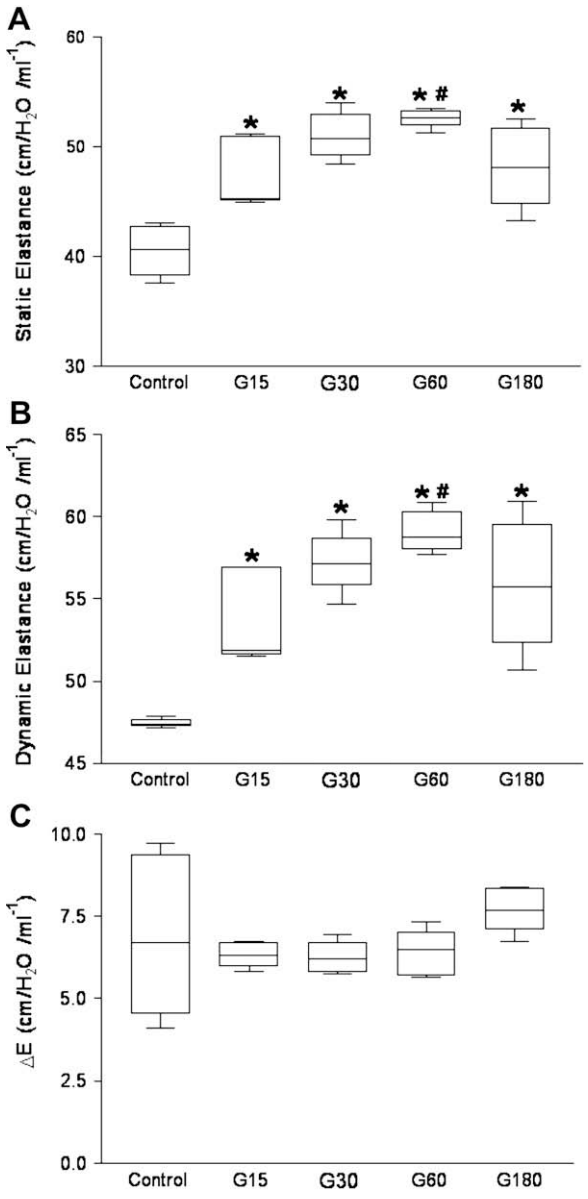

Fig. 1. Effect of TsV on mean values of static elastance (A), dynamic elastance (B) and elastance variation (C). The TsV (0.67 μg/g) was injected intraperitoneally. In (A) and (B), $^{*}p < 0.001$ when compared with the control group, and $^{\#}p < 0.05$ when compared with G15. In (C), no differences were found when comparing all groups ($p > 0.05$).

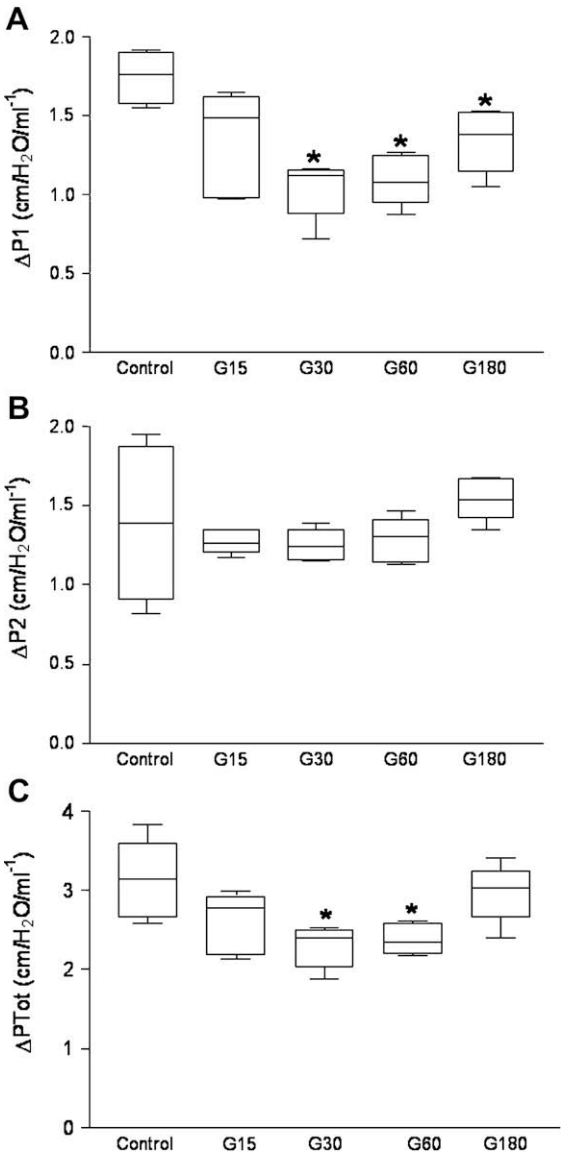

Fig. 2. Effect of TsV on mean values of ΔP_1 (A), ΔP_2 (B) and ΔP_{tot} (C). The TsV (0.67 μg/g) was injected intraperitoneally. In (A) and (C), $^{*}p < 0.001$ when compared with the control group. In (B), no differences were found when comparing all groups ($p > 0.05$).

increase in parenchymal and perivascular oedema after 15, 30, 60 and 180 min when compared to the control group. Fig. 4 shows the representative photomicrographs of lung histology stained with H/E focused on perivascular lung inflammation in the control and G15 group at 100× magnification as well as at 400× magnification (Panels A–D, respectively).

4. Discussion

This is the first study to evaluate the effects of intramuscular TsV injection at 15, 30, 60 and 180 min on the resistive and viscoelastic properties of the lungs and perivascular lung inflammation. The results demonstrate that TsV injection led to acute lung injury, characterised by increased static and dynamic elastance, decreased resistive properties and total resistive pressure variation of the lung tissue as well as an increase in perivascular lung infiltration by mononuclear and polymorphonuclear cells. These findings remained unaltered for 180 min.

The potential toxic effects of TsV are well documented, such as systemic inflammatory response, tachycardia, pancreatitis, myocardial damage, respiratory failure, epilepsy, renal oedema and multiple organ failure (Amitai, 1998; D'Suze et al., 2003; de Sousa Alves et al., 2005; Cupo et al., 2007). There are a reasonable number of studies

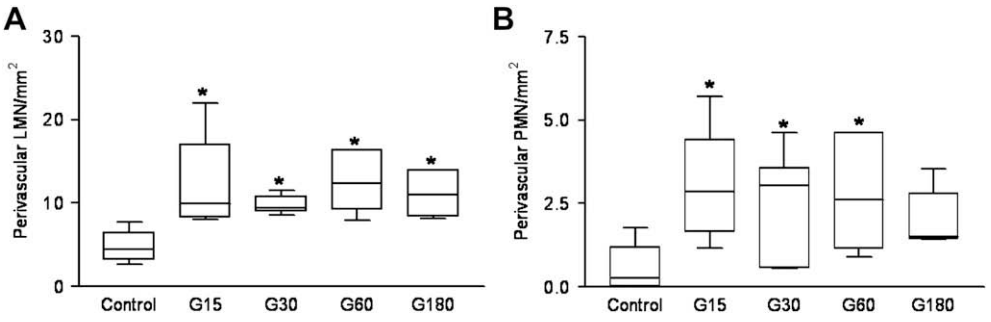

Fig. 3. Perivascular mononuclear and polymorphonuclear cells in the lungs of mice injected intraperitoneally with TsV. In (A) and (B), $^{*}p < 0.001$ when compared with the control group.

addressing the harmful effects of TsV on the lungs, particularly regarding the inflammatory response, including the release of pro-inflammatory cytokines, such as TNF-α and KC, as well as MPO activity (Comellas et al., 2003; Andrade et al., 2004, 2007; Coelho et al., 2007). However, only one previous study has evaluated lung function response (lung mechanics) following gamma fraction of TsV administration (Andrade et al., 2004). In the study, the authors demonstrated that intravenous gamma fraction of TsV administration resulted in decreased lung compliance (elastance) after 60 min (but not at 5, 15 and 30 min), with no change in lung resistance (Andrade et al., 2004). In contrast, the results of the present study demonstrated that the intramuscular administration of crude TsV resulted in increased elastance (static and dynamic) at 15, 30, 60 and 180 min following injection (Fig. 1A and B, respectively) and these values did not return to control values until 180 min.

Assuming that TsV leads to pulmonary alterations very similar to those observed in acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) (Rossi et al., 1974; D'Suze et al., 1999), we expected to find increased lung elastance, based on the many experimental studies on ARDS/ALI (Menezes et al., 2005; Card et al., 2006; Santos et al., 2006). In present study, there were some common histopathological findings (intense neutrophilic inflammation)

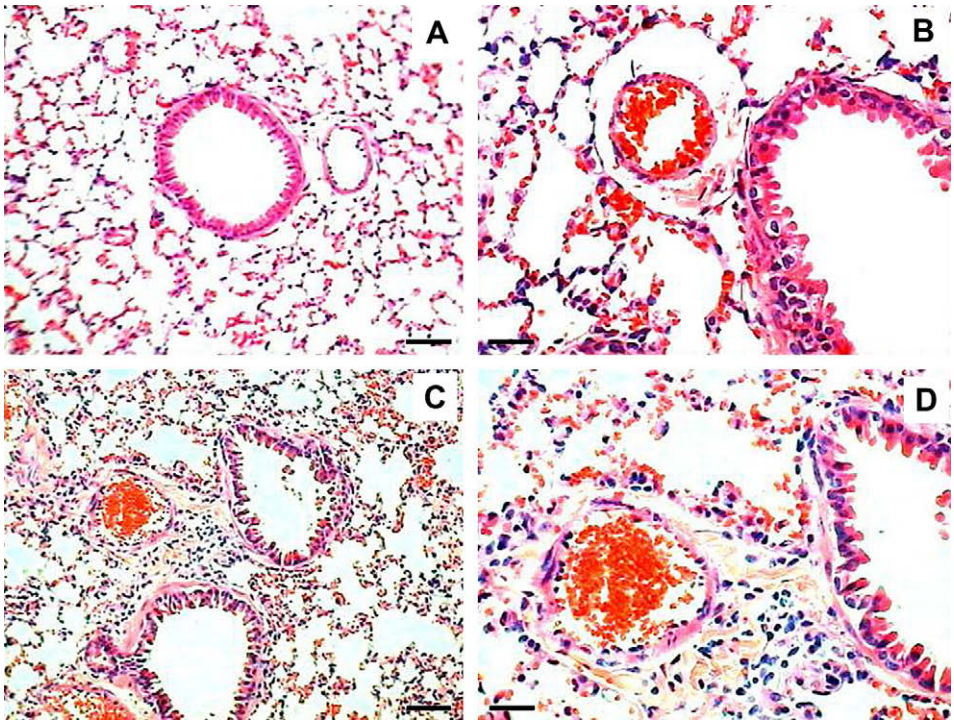

Fig. 4. Representative photomicrographs of lung histology stained with H/E focused on perivascular lung inflammation. Panel (A) – control group shows normal morphological appearance of lung tissue, with normal vascular architecture and few leukocytes in the perivascular space (at 100× magnification). Panel (B) – the same photomicrograph viewed at 400× magnification. Panel (C) – G15 corresponding to 15 min after TsV injection, presenting increased density of polymorphonuclear cells in the perivascular space (at 100× magnification). Panel (D), the same photomicrograph viewed at 400× magnification. In (A) and (B), scale bar = 100 μm. In (C) and (D), scale bar = 25 μm.

between the lung effects of *T. serrulatus* venom and ARDS/ALI as well as similar alterations in lung mechanics, i.e. increased elastance. However, it should be stressed that different results regarding elastance may be found depending on the experimental model used for inducing acute lung injury and the different methods used for the analysis of lung mechanics (Andrade et al., 2004; Card et al., 2006; Santos et al., 2006). Furthermore, discrepancies between the results of the present study and the study conducted by Andrade et al. (2004) could be expected due to the fact that we used crude TsV and Andrade et al. (2004) used a gamma fraction of TsV. A possible hypothesis underlying TsV-induced decreased elastance to be investigated in further studies could be the fact that TsV contains gelatinolytic enzymes (Almeida et al., 2002), which may lead to the degradation of extra-cellular matrix proteins in the airways. We might also hypothesise that activated neutrophils and macrophages (as found in the present study) release increased amounts of matrix metalloproteases (MMPs), which are capable of degrading extra-cellular matrix proteins, thereby leading to decreased elastance (Greenlee et al., 2007; Ra and Parks, 2007).

The present study also demonstrated that TsV intramuscular injection led to decreased ΔP_1 values at 30, 60 and 180 min (Fig. 2A), suggesting a decrease in airway resistance. This finding is supported by a study by Card et al. (2006), who demonstrated that an experimental ARDS/ALI model affected both proximal and distal airways as well as the distal parenchyma, while lipopolysaccharide increased airway resistance (Card et al., 2006). However, no evaluation of airway inflammation was carried out in the present study, but rather perivascular lung inflammation. Therefore, we cannot affirm that TsV decreased airway resistance as a result of an inflammatory airway process. Our results also demonstrated that TsV decreased ΔP_{tot} values at 30 and 60 min following injection (Fig. 2C), suggesting decreased total pressure variation in the lung despite the greater pressure generated by elastic forces (Nonaka et al., 2008). Along with other causes, these effects may be associated with increased alveolar oedema (Menezes et al., 2005; Santos et al., 2006). Therefore, the results of the present study show that TsV led to mechanical alterations in pulmonary tissue and pulmonary pressure and these effects were not reversible until 180 min.

The effects of TsV on lung mechanics may also be attributed to the release of inflammatory mediators by inflammatory cells activated by TsV (Coelho et al., 2007). The main inflammatory mediators released following the administration of scorpion venom in mice was due to the activation of the inflammatory cascade and release of lipid-derived inflammation mediators, including platelet activation factor (PAF), leukotrienes and prostaglandins (De Matos et al., 1997). However, we could not explain how TsV (a neurotoxin) could activate the inflammatory cascade and consequently induce pulmonary oedema. One distinct possibility has been raised from our studies on the role of the tachykinin NK1 receptor antagonists in mice. These studies demonstrated that antagonists of the tachykinin NK1 receptor, in which substance P is the main ligand, virtually abolished lung oedema and lethality induced by TsV (Matos et al., 1999). However, this is just a hypothesis, as no measurements of the levels of substance P or the expression of NK1 receptor were performed. Moreover, it is not possible to exclude the possibility that the presence of other neurotoxic proteins could be mediating these effects, including a large number of alpha toxins, which have an antagonist effect induced by beta toxins (gamma toxin) found in TsV.

TsV also releases noradrenaline from adrenergic neurons and, after persistent activation of adrenergic receptors, vasoconstriction spontaneously fades out through a myogenic mechanism that controls vessel flow, namely, vascular escape. This effect must be an important mechanism for the renal and vascular effect induced by TsV. Therefore, these effects should be further investigated as part of the mechanisms involved in the effects of TsV on the lungs.

In conclusion, the results of present study permit us to affirm that *T. serrulatus* venom provokes mechanical alterations in pulmonary tissue and pressure as well as intense perivascular lung infiltration by mono and polymorphonuclear cells, and these effects remain unaltered for as much as 180 min after TsV injection. Our results suggest that the physiological modification induced by this venom is a multi-factor event induced by the toxins present in the TsV.

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Conflicts of interest

No financial or other potential conflicts of interest exist.

References

Almeida, F.M., Pimenta, A.M., De Figueiredo, S.G., Santoro, M.M., Martin-Eauclaire, M.F., Diniz, C.R., De Lima, M.E., 2002. Enzymes with gelatinolytic activity can be found in *Tityus bahiensis* and *Tityus serrulatus* venoms. Toxicon 40, 1041–1045.

Amaral, C.F.S., Rezende, N.A., Freire-Maia, L., 1993. Acute pulmonary edema after *Tityus serrulatus* scorpion sting in children. Am. J. Cardiol. 71, 242–245.

Amitai, Y., 1998. Clinical manifestations and management of scorpion envenomation. Public Health Rev. 26, 257–263.

Andrade, M.V., Caramez, M.P., Abreu, E.M., Dolnikoff, M., Omar, E.D., Velasco, I.T., Cunha-Melo, J.R., 2004. Lung compliance, plasma electrolyte levels and acid-base balance are affected by scorpion envenomation in anesthetized rats under mechanical ventilation. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 138, 97–104.

Andrade, M.V., Lisboa, F.A., Portugal, A.L., Arantes, R.M., Cunha-Melo, J.R., 2007. Scorpion venom increases mRNA expression of lung cytokines. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 146, 581–587.

Bates, J.H.T., Rossi, A., Milic-Emili, J., 1985. Analysis of the behavior of the respiratory system with constant inspiratory flow. J. Appl. Physiol. 58, 1840–1848.

Brazilian National Foundation of Health, 1998. Manual de Diagnóstico e Tratamento de Acidentes por Animais Peçonhentos. Ministério da Saúde, Brasília, p. 141.

Card, J.W., Carey, M.A., Bradbury, J.A., DeGraff, L.M., Morgan, D.L., Moorman, M.P., Flake, G.P., Zeldin, D.C., 2006. Gender differences in murine airway responsiveness and lipopolysaccharide-induced inflammation. J. Immunol. 177, 621–630.

Chang, H.K., Mortola, J.P., 1981. Fluid dynamic factors in tracheal pressure measurement. J. Appl. Physiol. 51, 218–225.

Comellas, A.P., Pesce, L.M., Azzam, Z., Saldías, F.J., Sznajder, J.I., 2003. Scorpion venom decreases lung liquid clearance in rats. Am. J. Respir. Crit. Care Med. 167, 1064–1067.

Coelho, F.M., Pessini, A.C., Coelho, A.M., Pinho, V.S., Souza, D.G., Arantes, E.C., Teixeira, M.M., Teixeira, A.L., 2007. Platelet activating factor receptors drive CXC chemokine production, neutrophil influx and edema formation in the lungs of mice injected with *Tityus serrulatus* venom. Toxicon 50, 420–427.

Corrêa, M.M., Sampaio, S.V., Lopes, R.A., Mancuso, L.C., Cunha, O.A., Franco, J.J., Giglio, J.R., 1997. Biochemical and histopathological alterations induced in rats by *Tityus serrulatus* scorpion venom and its major neurotoxin tityustoxin-I. Toxicon 35, 1053–1067.

Cupo, P., Figueiredo, A.B., Filho, A.P., Pintya, A.O., Tavares Jr., G.A., Caligaris, F., Marin-Neto, J.A., Hering, S.E., Simões, M.V., 2007. Acute left ventricular dysfunction of severe scorpion envenomation is related to myocardial perfusion disturbance. Int. J. Cardiol. 116, 98–106.

D'Suze, G., Comellas, A., Pesce, L., Sevcik, C., Sanches-de-León, R., 1999. *Tityus discrepans* venom produces a respiratory distress syndrome in rabbits through an indirect mechanism. Toxicon 37, 173–180.

D'Suze, G., Moncada, S., Gonzalez, C., Sevicik, C., Aguilar, V., Alagon, A., 2003. Relationship between plasmatic levels of various cytokines, tumour necrosis factor, enzymes, glucose and venom concentration following *Tityus* scorpion sting. Toxicon 41, 367–375.

De Matos, I.M., Rocha, O.A., Leite, R., Freire-Maia, L., 1997. Lung edema induced by *Tityus serrulatus* scorpion venom in the rat. Comp. Biochem. Physiol. 118, 143–148.

de Sousa Alves, R., do Nascimento, N.R., Barbosa, P.S., Kerntopf, M.R., Lessa, L.M., de Sousa, C.M., Martins, R.D., Sousa, D.F., de Queiroz, M.G., Toyama, M.H., Fonteles, M.C., Martins, A.M., Monteiro, H.S., 2005. Renal effects and vascular reactivity induced by *Tityus serrulatus* venom. Toxicon 46, 271–276.

Greenlee, K.J., Werb, Z., Kheradmand, F., 2007. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. Physiol. Rev. 87, 69–98.

Guidine, P.A., Assumpção, G., Moraes-Santos, T., Massensini, A.R., Chianca Jr., D.A., Moraes, M.F., 2008. Dose-dependent effect of carbamazepine on weanling rats submitted to subcutaneous injection of tityustoxin. Neurosci. Lett. 433, 170–173.

Ismail, M., Abd-Elsalam, A., Morad, M., 1990. Do changes in body temperature following envenomation by the scorpion *Leiurus quinquestriatus* influence the course of toxicity? Toxicon 23, 1265–1284.

Ismail, M., Abd-Elsalam, A., 1988. Are the toxicological effects of scorpion envenomation related to tissue venom concentration? Toxicon 26, 233–236.

Matos, I.M., Souza, D.G., Seabra, D.G., Freire-Maia, L., Teixeira, M.M., 1999. Effects of tachykinin NK1 or PAF receptor blockade on the lung injury induced by scorpion venom in rats. Eur. J. Pharmacol. 376, 293–300.

Mall, M.A., Harkema, J.R., Trojanek, J.B., Treis, D., Livraghi, A., Schubert, S., Zhou, Z., Kreda, S.M., Tilley, S.L., Hudson, E.J., O'Neal, W.K., Boucher, R.C., 2008. Development of chronic bronchitis and emphysema in beta-epithelial Na^+ channel-overexpressing mice. Am. J. Respir. Crit. Care Med. 177, 730–742.

Menezes, S.L., Bozza, P.T., Neto, H.C., Laranjeira, A.P., Negri, E.M., Capelozzi, V.L., Zin, W.A., Rocco, P.R., 2005. Pulmonary and extrapulmonary acute lung injury: inflammatory and ultrastructural analyses. J. Appl. Physiol. 98, 1777–1783.

Mesquita, M.B.S., Moraes-Santos, T., Moraes, M.D.F., 2002. Phenobarbital blocks the lung edema induced by centrally injected tityustoxin in adult Wistar rats. Neurosci. Lett. 332, 119–122.

Mortoloa, J.P., Naworaj, A., 1983. Two-sidearm tracheal cannula for respiratory airflow measurements in small animals. J. Appl. Physiol. 55, 250–253.

Murthy, R.K., Haghnazari, L., 1999. The blood levels of glucagon, cortisol and insulin following the injection of venom by scorpion (*Mesobuthus tamulus concanensis*, Pocock) in dogs. J. Venom. Anim. Toxins 5, 47–55.

Nonaka, P.N., Amorim, C.F., Paneque Peres, A.C., Silva, C.A.E., Zamuner, S.R., Ribeiro, W., Cogo, J.C., Vieira, R.P., Dolhnikoff, M., de Oliveira, L.V., 2008. Pulmonary mechanic and lung histology injury induced by *Crotalus durissus terrificus* snake venom. Toxicon 51, 1158–1166.

Nunan, E.A., Arya, V., Hochhaus, G., Cardoso, V.N., Moraes-Santos, T., 2004. Age effects on the pharmacokinetics of tityustoxin from *Tityus serrulatus* scorpion venom in rats. Braz. J. Med. Biol. Res. 37, 385–390.

Ra, H.J., Parks, W.C., 2007. Control of matrix metalloproteinase catalytic activity. Matrix Biol. 26, 587–596.

Rossi, M.A., Ferreira, A.L., Paiva, S.M., 1974. Fine structures of pulmonary changes induced by Brazilian scorpion venom. Arch. Pathol. 97, 284–288.

Saldiva, P.H.N., Zin, W.A., Santos, R.L.B., Eidelman, D.H., Milic-Emili, J., 1992. Alveolar pressure measurement in open-chest rats. J. Appl. Physiol. 72, 302–306.

Santos, F.B., Nagato, L.K., Boechem, N.M., Negri, E.M., Guimarães, A., Capelozzi, V.L., Faffe, D.S., Zin, W.A., Rocco, P.R., 2006. Time course of lung parenchyma remodeling in pulmonary and extrapulmonary acute lung injury. J. Appl. Physiol. 100, 98–106.

Silveira, K.S.O., Boechem, N.T., Nascimento, S.M., Murakami, Y.L.B., Barboza, A.P.B., Melo, P.A., Castro, P., Moraes, V.L.G., Rocco, P.R.M., Zin, W.A., 2004. Pulmonary mechanics and lung histology in acute lung injury induced by *Bothrops jararaca* venom. Respir. Physiol. Neurobiol. 139, 167–177.

Sofer, S., Gueron, M., 1988. Respiratory failure in children following envenomation by the scorpion *Leiurus quinquestriatus*: hemodynamic and neurological aspects. Toxicon 26, 931–939.

Vasconcelos, F., Sampaio, S.V., Garófalo, M.A., Guimarães, L.F., Giglio, J.R., Arantes, E.C., 2004. Insulin-like effects of *Bauhinia forficata* aqueous extract upon *Tityus serrulatus* scorpion envenoming. J. Ethnopharmacol. 95, 385–392.

Vieira, R.P., Claudino, R.C., Duarte, A.C., Santos, A.B., Perini, A., Faria Neto, H.C., Mauad, T., Martins, M.A., Dolhnikoff, M., Carvalho, C.R., 2007a. Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice. Am. J. Respir. Crit. Care Med. 176, 871–877.

Vieira, R.P., Duarte, A.C., Claudino, R.C., Perini, A., Santos, A.B., Moriya, H.T., Arantes-Costa, F.M., Martins, M.A., Carvalho, C.R., Dolhnikoff, M., 2007b. Creatine supplementation exacerbates allergic lung inflammation and airway remodeling in mice. Am. J. Respir. Cell. Mol. Biol. 37, 660–667.

Vieira, R.P., Duarte, A.C., Andrade, V.F., Santos, A.B.G., Mauad, T., Martins, M.A., Dolhnikoff, M., Carvalho, C.R.F., 2008. Aerobic conditioning and allergic pulmonary inflammation in mice II: effects on lung vascular and parenchymal inflammation and remodeling. Am. J. Physiol. Lung Cell. Mol. Physiol. 295, L670–L679.

Xisto, D.G., Farias, L.L., Ferreira, H.C., Picanço, M.R., Amitrano, D., Lapa e Silva, J.R., Negri, E.M., Mauad, T., Carnielli, D., Silva, L.F., Capelozzi, V.L., Faffe, D.S., Zin, W.A., Rocco, P.R., 2005. Lung parenchyma remodeling in a murine model of chronic allergic inflammation. Am. J. Respir. Crit. Care Med. 171, 829–837.