ELSEVIER

Toxicon 51 (2008) 1158–1166

TOXICON

www.elsevier.com/locate/toxicon

Pulmonary mechanic and lung histology injury induced by *Crotalus durissus terrificus* snake venom

Paula Naomi Nonaka[a], César Ferreira Amorim[a], Ana Claudia Paneque Peres[a], César Augusto Melo e Silva[b], Stella R. Zamuner[a], Wellington Ribeiro[a], José Carlos Cogo[a], Rodolfo P. Vieira[c], Marisa Dolhnikoff[c], Luis Vicente Franco de Oliveira[d,*]

[a]*Laboratory of Physiology and Pharmacodynamics, Institute of Research and Development—IP&D, University of Vale do Paraiba—UNIVAP, Sao Jose dos Campos, SP, Brazil*

[b]*Laboratory of Respiratory Physiology, University Hospital, University of Brasília—UnB, Brasília, DF, Brazil*

[c]*Department of Pathology, School of Medicine, University of Sao Paulo—USP, São Paulo, Brazil*

[d]*Laboratory of Respiratory Physiology, Rehabilitation Sciences Master's Program, University of Nove de Julho—UNINOVE, Av. Francisco Matarazo 612, Água Branca, CEP 05.001-100, São Paulo, SP, Brazil*

Received 27 September 2007; received in revised form 24 December 2007; accepted 1 February 2008

Abstract

In the present work we investigated the effects of *Crotalus durissus terrificus* venom (CdtV) on the pulmonary mechanic events [static and dynamic elastance, resistive ($\Delta P1$) and viscoelastic pressures ($\Delta P2$)] and histology after intramuscular injection of saline solution (control) or venom (0.6 μg/g). The static and dynamic elastance values were increased significantly after 3 h of venom inoculation, but were reduced at control values in the other periods studied. The $\Delta P1$ values that correspond to the resistive properties of lung tissue presented a significant increase after 6 h of CdtV injection, reducing to basal levels 12 h after the venom injection. In $\Delta P2$ analysis, correspondent to viscoelastic components, an increase occurred 12 h after the venom injection, returning to control values at 24 h. CdtV also caused an increase of leukocytes recruitment (3–24 h) to the airways wall as well as to the lung parenchyma. In conclusion, *C. durissus terrificus* rattlesnake venom leads to lung injury which is reverted, after 24 h of inoculation.

Keywords: *Crotalus durissus terrificus*; Inflammation; Respiratory system; Lung mechanics

1. Introduction

Snakes of the genus *Crotalus* are responsible for most ophidian accident with high level of mortality that occurs in Brazil (Jorge and Ribeiro, 1992). The venom of the South American rattlesnake *Crotalus durissus terrificus* (CdtV) is composed by a powerful neurotoxin, crotoxin, that accounts for approximately 50% of the weight of the venom and is responsible for most of the symptoms observed following envenomation of the crude venom (Toyama et al., 2003; Santos et al., 2005). It has been shown

Abbreviations: CdtV, *Crotalus durissus terrificus* venom.

*Corresponding author. Tel.: +12 3947 1144.
E-mail address: oliveira.lvf@uninove.br (L.V.F. de Oliveira).

0041-0101/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2008.02.006

that CdtV induces neuromuscular blockade, mionecrose and edema (Santoro et al., 1999; Toyama et al., 2003; Oguiura et al., 2005). Also, according to Vital Brazil (1972), the envenomation caused by CdtV frequently causes acute respiratory insufficiency related to the neuromuscular blockade. However, specific studies on the mechanical respiratory system caused by CdtV are scarce and show only the pulmonary sprouting of edema as respiratory consequence (Santoro et al., 1999; Santos et al., 2005). There is no study in the literature involving the measurement of the mechanical properties that allow studying the behavior of the respiratory system in reply to the injury generated by this venom. Furthermore, Brooks et al. (2002) described that the study with respiratory compromise after rattlesnake envenomation is an uncommon, but yet potentially lethal complication and is rarely reported in the literature.

The resistance of the pulmonary airways to airflow is an important determinant of lung function, and its measurement is central to the study of respiratory mechanisms and the diagnosis of obstructive lung disease (Bates et al., 1988). The pulmonary ventilation involves the movement of the respiratory system, which requires the accomplishment of a mechanical work to win opposition forces. These include the elastic, resistive and viscoelastic components of the lung tissue and the chest wall (Zin and Rocco, 1999). The study of lung mechanics properties also have been related to lung histological alterations, as formation of edema, hemorrhage, inflammation and increased deposition of matrix extra-cellular proteins (Santos et al., 2006). Therefore, to evaluate the effects of CdtV on the lung mechanics and also its effects on the lung histology could be helpful on the elucidation of harmful effects of CdtV envenomation.

In the present work we investigate the behavior of the mechanical properties of the respiratory system, the characterization of the pulmonary structures and the quantification of the inflammatory process generated by the action of the CdtV. For this purpose, lung elastic, resistive and viscoelastic mechanical changes were determined at 3, 6, 12 and 24 h after intramuscular injection of CdtV in mice and the results were related to data gathered from pulmonary histopathology.

2. Material and methods

2.1. Venom

Lyophilizied crude CdtV was obtained from the Centro de Estudos da Natureza (CEN)—UNIVAP, São Jose dos Campos, Brazil, and stored at −20 °C. Venom was dissolved in sterile physiological saline (0.9%, w/v NaCl solution) at the moment of use.

2.2. Animals

Male Swiss mice (18–20 g) were kept housed in temperature-controlled, relative humidity of 65.3 ± 0.9% and 12 h dark photo-period (lights on at 6:00 h a.m.), received water and food ad libitum. The animals and research protocols used in this study are in accordance with guidelines of the Ethical Committee for use of animals of Universidade do Vale do Paraíba-CEP, protocol number L057/2005.

2.3. Letal dose (LD_{50})

The mean lethal dose of intramuscular injection of CdtV in mice is 0.6 μg/g body weight (BW). This LD_{50} had been previously determined in our laboratory by intramuscular injection of five different doses (0.5, 0.6, 0.8 and 1.10 μg/g BW) in five groups of mice each, and recording the death during 72 h.

2.4. Experimental procedure

CdtV (0.6 μg/g) dissolved in 50 μl of sterile saline was injected intramuscularly (i.m.). Control animals received 50 μl of sterile solution alone. After 3, 6, 12 and 24 h of venom injection the animals were sedated [pentobarbital sodium (0.7 μl) and a snugly fitting cannula (1.1 mm ID) was introduced into the trachea and then connected to a mechanical ventilation (*Samay MVR16xp*, Montevideu, Uruguai) computer controlled]. The animals rested in the supine position on a surgical table.

The anterior chest wall was surgically removed. Mechanical ventilation with a frequency of 100 breaths/min, a tidal volume of 0.2 ml, and a adequate amount of positive end-expiratory pressure (PEEP = 2 cmH_2O) was applied right before the pleural cavity was entered. The PEEP level was determined as follows: before the pleural space was opened, the ventilator was disconnected at end expiration, and the airways were occluded. After pleural incision, there was an increase in transpulmonary pressure (PL) that corresponded to the elastic recoil pressure of the lung at relaxation

volume. Thereafter, the same pressure was applied to the lung as PEEP (Silveira et al., 2004).

A pneumotachograph (Mortola and Noworaj, 1983) was connected to the tracheal cannula for the measurements of airflow (V'), and changes in lung volume (V_T). The flow resistance of the equipment (R_{eq}), tracheal cannula included, was constant up to flow rates of 26 ml/s, and amounted to 0.12 cmH_2O/ml s. Equipment resistive pressure ($R_{eq}V'$) was subtracted from pulmonary resistive pressure so that the present results represent intrinsic values. Because abrupt changes of diameter were not present in our circuit, errors of measurement of flow resistance were avoided (Chang and Mortola, 1981). Flow and pressure signals were measured by transducers connected to the pneumotachograph and then to an *EMGsystem do Brasil* signal conditioner 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, OH, USA).

2.5. Measurement of pulmonary mechanics

Pulmonary mechanics were measured from end-inspiratory occlusions after constant flow inflations (Bates et al., 1985; Silveira et al., 2004). In an open chest preparation, tracheal pressure reflects transpulmonary pressure (PL). After end-inspiratory occlusion, there is an initial fast drop in PL ($\Delta P1$) from the preocclusion value down to an inflection point (P_i) followed by a slow pressure decay ($\Delta P2$) until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the lung (P_{el}). $\Delta P1$ selectively reflects pressure dissipated against pulmonary resistance in normal animals and humans, and $\Delta P2$ reflects viscoelastic properties (stress relaxation) and/or in homogeneities of lung tissues together with a tiny contribution of pendelluft in normal situations (Bates et al., 1988; Saldiva et al., 1992; Silveira et al., 2004). Total pressure drop (ΔP_{tot}) is equal to the sum of $\Delta P1$ and $\Delta P2$. Lung static elastance (E_{st}) was calculated by dividing P_{el} by V_T. Dynamic elastance of the lung (E_{dyn}) was obtained by dividing P_i by V_T. ΔE was calculated as the difference E_{dyn}–E_{st}. Pulmonary mechanics measurements were performed 10–15 times in each animal. The experiments did not last more than 40 min. Data analysis was performed with ANADAT software (RHT InfoData, Montreal, Canada).

2.6. Lung histology

Histopathological analysis was performed in six to nine excised lungs of each group. The abdominal aorta and vena cava were then sectioned, yielding a massive hemorrhage that quickly killed the animals. Immediately after the end of mechanics measurements, 0.3 ml of formalin was instilled into the lungs. The lungs were surgically removed en bloc and fixed in formalin for 48 h. The slices (5-μm thick) were stained with haematoxylin–eosin and examined by a pathologist without any knowledge of the treatment group. Morphometric analysis of peribronchial (five airways per animal) and parenchymal (20 fields per animal) inflammation were performed through of differential cell counting (mononuclear and polymorphonuclear) using a count point technique (Lançcas et al., 2006; Vieira et al., 2007). Using a 100-point grid with a known area (62,500 μm^2 at a 400 × magnification) attached to the microscope ocular, the number of points hitting the outer area of the airway wall (located between the external limit of smooth muscle layer and the adventitia) was computed. The airway area in each field was calculated according to the number of points hitting the airway, as a proportion of the total grid area. The number of mononuclear and polymorphonuclear cells was counted. For each cell type, cellular density was determined as the number of positive cells in each field, divided by tissue area. Measurements were expressed as cells/μm^2. The results were then transformed to cells/mm^2 by adjusting the units (Lançcas et al., 2006; Vieira et al., 2007). In the alveolar parenchyma, we counted the number of points hitting alveolar tissue in each field and the number of mononuclear and polymorphonuclear cells within the alveolar septa. Cellular density was determined as the number of inflammatory cells in each field divided by tissue area (Lançcas et al., 2006). The fractional area of normal, collapsed and hyperinflated (structures with a distinct morphology from that of alveoli and wider than 120 μm) alveoli were computed in random, non-coincident, ten microscopic fields by the point-counting technique at a 400 × magnification (Weibel, 1990; Santos et al., 2006).

2.7. Statistical analysis

To compare the results gathered from each group, initially the normality of the data (Kolmogorow–Smirnov test with Lilliefor's correction), and the

homogeneity of variances (Levene median test) were evaluated. If both conditions were satisfied, one-way ANOVA was used. In the negative case, Kruskal–Wallis ANOVA was selected instead. Two-way ANOVA was used for comparison of venom effects over time, using the dose of venom and the time after injection as the two factors for analysis. If multiple comparisons were required, Tukey test was applied. The significance level was always set at 5%.

3. Results

3.1. Pulmonary mechanic

For the execution of the analysis of the pulmonary mechanical properties in this study the measurement of flow variance, volume and PEEP had been doing. Table 1 show that there is no difference in the parameter analyzed among animals as well as groups, and they were not superior to 10% and 30%, respectively.

3.2. Elastic, resistive and viscoelastic properties

After the application of the venom, the values of static and dynamic elastance increased significantly 3 h after the venom injection, returned to basal levels 6 h after, and maintained the basal levels over the following 12–24 h (Fig. 1A and B). The variation of pulmonary elastance, translated for the difference between dynamic elastance and the static elastance, shows a gradual increase, with a significant peak 12 h after the venom injection, not returning to control values until 24 h (Fig. 1C).

Fig. 2A shows the values of $\Delta P1$, which represent resistance properties of the pulmonary tissue. CdtV induced a significant increase in $\Delta P1$ between 3 and 12 h after the venom injection as compared with

Table 1
Control, injected with saline; V3, 3 h after CdtV (0.6 μg/g) injection; V6, 6 h after CdtV (0.6 μg/g) injection; V12, 12 h after CdtV (0.6 μg/g) injection; V24, 24 h after CdtV (0.6 μg/g) injection; PEEP, positive end-expiratory pressure

$N = 30$	Flow (ml/s)	Volume (ml)	PEEP (cmH_2O)
Control	1.20±0.23	0.21±0.01	2.49±0.08
G3	1.29±0.17	0.21±0.01	2.41±0.12
G6	1.13±0.05	0.20±0.00	2.17±0.06
G12	1.09±0.00	0.20±0.00	2.52±0.00
G24	1.09±0.00	0.20±0.00	2.52±0.00

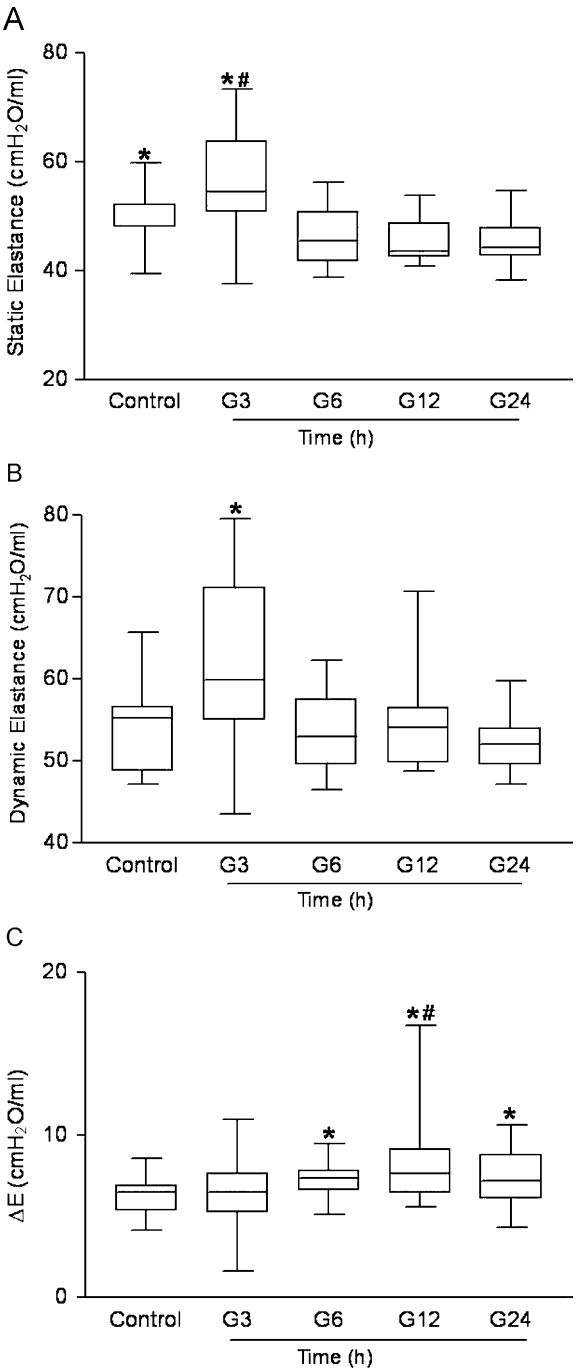

Fig. 1. Effect of *Crotalus durissus terrificus* venom on mean values of static elastance (A), dynamic elastance (B) and of mean values elastance variation (C). The CdtV (0.6 μg/g) was injected intramuscularly. At different times after venom injection the values of elastance was measured as described in Section 2. Results are expressed as box plot from five animals in each group. In (A) $^{*}p<0.01$ when compared with G3, G6 and G12 group and $^{\#}p<0.01$ when compared with all groups. In (B) $^{*}p<0.001$ when compared with all groups. In (C) $^{*}p<0.05$ when compared with Control group and $^{\#}p<0.05$ when compared with Control and G3 group.

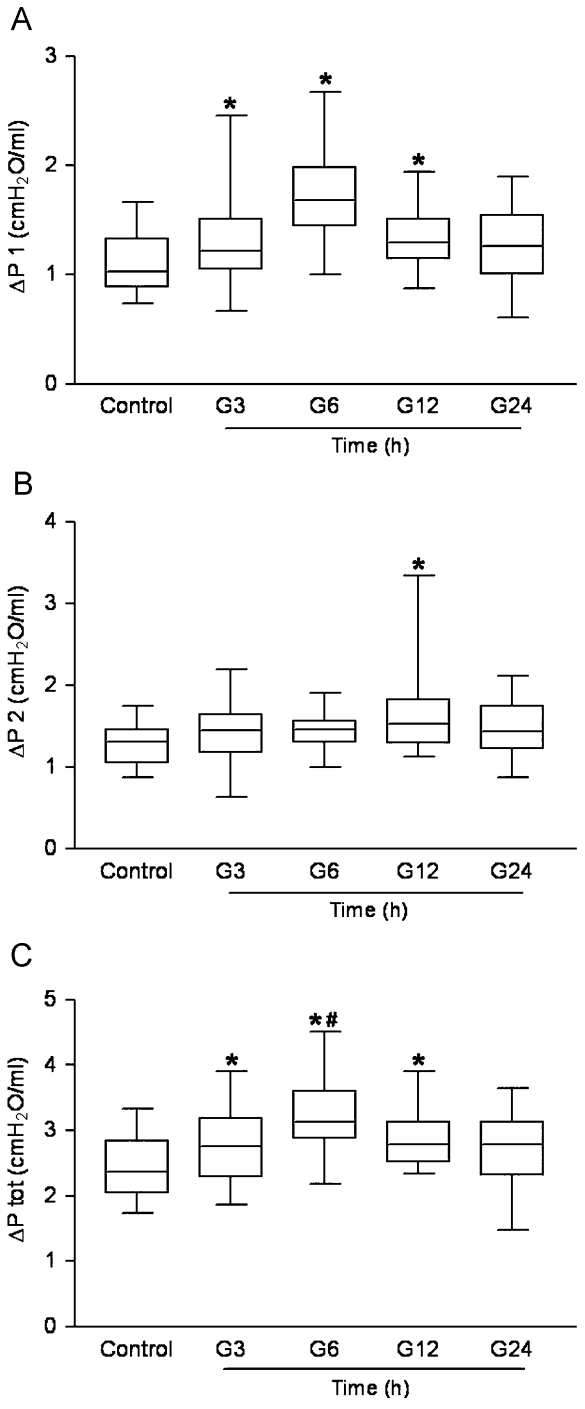

Fig. 2. Effect of *Crotalus durissus terrificus* venom on mean values of $\Delta P1$ (A), $\Delta P2$ (B) and of ΔP_{tot} (C). The CdtV (0.6 μg/g) was injected intramuscularly. At different times after venom injection the $\Delta P1$, $\Delta P2$ and ΔP_{tot} values were measured as described in Section 2. Results as expressed as box plot from five animals in each group. In (A) $^{*}p<0.01$ when compared with control group. In (B) $^{*}p<0.05$ when compared with control group. In (C) $^{*}p<0.01$ when compared with Control group and $^{\#}p<0.01$ when compared with all groups.

control values. The peak of $\Delta P1$ occurred 6 h after the CdtV injection, returning to the basal values at 24 h. CdtV induced a gradual increase in $\Delta P2$ values, referring to the viscoelastic properties, with peak 12 h after venom injection and returning to control values at 24 h (Fig. 2B).

3.3. Total pressure variation

To further explore the involvement in CdtV-induced lung injury, the analysis of total pressure variation in the lung, represented for ΔP_{tot}, was analyzed. Fig. 2C shows a significant increase in ΔP_{tot} at 3, 6 and 12 h after venom injection when compared to control values. The peak increase in ΔP_{tot} occurred at 6 h after CdtV injection, returning to control values at 24 h (Fig. 2C).

3.4. Histology

Qualitative and quantitative analysis was carried through. In the qualitative analysis of the pulmonary tissue after the induction of the injury process, the presence of perivascular edema 6 h after venom injection was observed. Diffuses hemorrhage in the lung parenchyma was seen 12 h after CdtV injection. The quantitative analysis of the pulmonary inflammation was assessed as the density of mononuclear and polymorphnuclear cells in the peribronchial lung compartment (around of the wall of the airways) and in the alveolar parenchyma, which showed a significant increase after 3 and 6 h, returning to control values 24 h after the venom injection (Figs. 3 and 4).

4. Discussion

Our study analyzed, by the first time, the effects of CdtV on the lung mechanical properties and histology. The study demonstrated that CdtV increases static and dynamic elastance, pulmonary tissue resistance and the inflammation in the airways and in the alveolar parenchyma at different times.

The effect of CdtV in the presynaptic motor endplate of the neuromuscular junction is well known. Also, the alterations in kidneys, liver and local action of this venom is well studied (Bancher et al., 1973; Barraviera, 1990, 1993; Santoro et al., 1999; Toyama et al., 2003). Although little is known about the actions of this venom in the respiratory system, it is presented only in studies of human

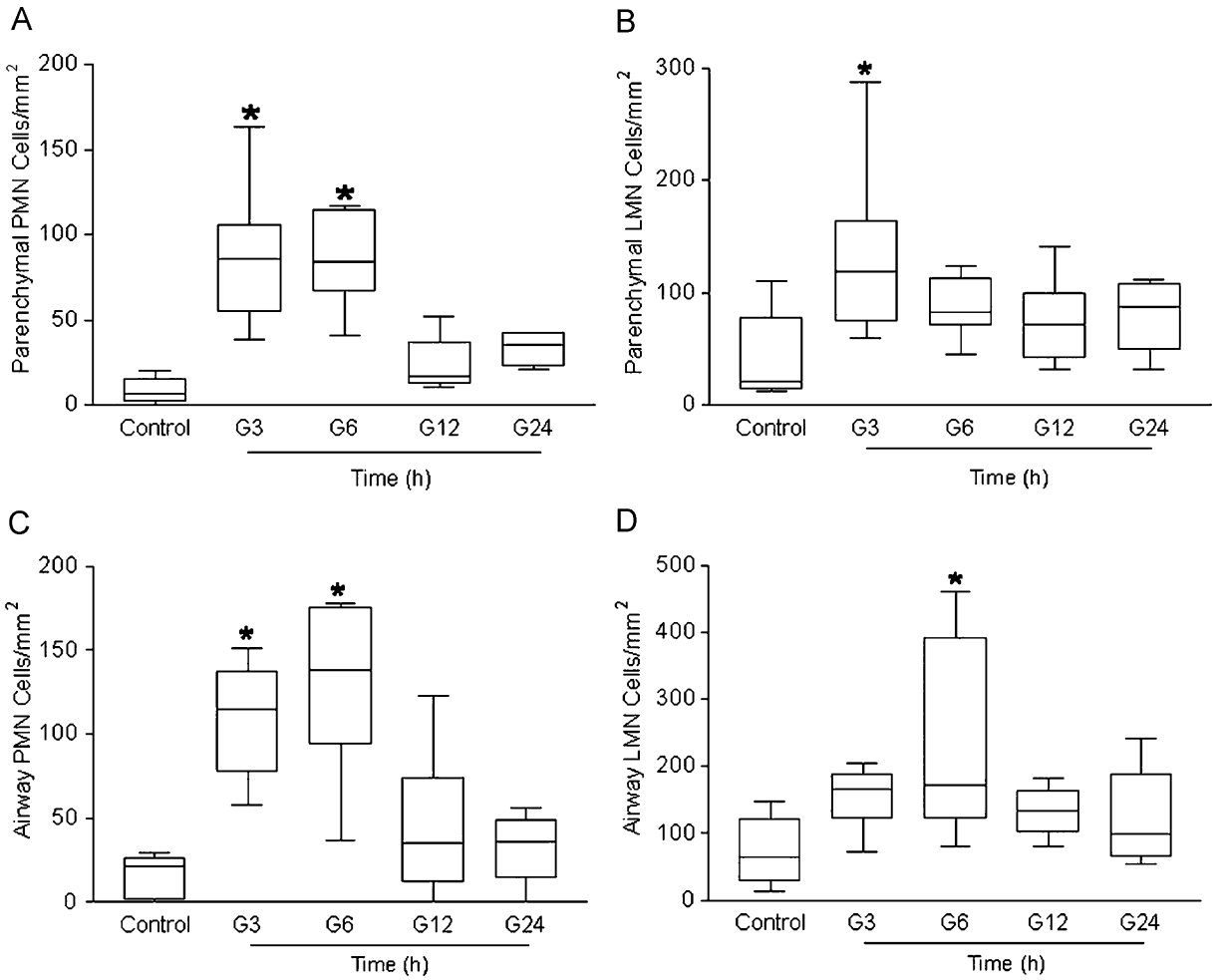

Fig. 3. Total leukocyte (mononuclear and polymorphonuclear cells) in lung of mice injected intramuscularly with *Crotalus durissus terrificus* venom. The CdtV (0.6 μg/g) was injected intramuscularly. The differential cell counting was performed in the peribronchial lung compartment and in the alveolar parenchyma, as described in Section 2. In (A) and (B), respectively, we show the polymorphonuclear and mononuclear cells in the peribronchial compartment. In (C) and (D), respectively, we show the polymorphonuclear and mononuclear cells in the alveolar parenchyma. Results as expressed as box plot from six to nine animal in each group. $*p<0.05$ compared to control.

cases (Amaral et al., 1991). In general, the respiratory complication after CdtV accident installs in the first 24 h (Amaral et al., 1991). Clinical observation in patients bitten by C. *durissus terrificus* showed alterations in the respiratory system that include the formation of atelectasis, the developing of a restrictive ventilation standard due to a compromised respiratory muscle, intense to the point to produce acute respiratory failure associate to the accumulation of secretions in the airways, pulmonary congestion and edema (Amaral et al., 1991). Such physiopathology behavior suggests consequent alteration of the elastic and viscoelastic pulmonary mechanical properties. In our study, the values of $\Delta P1$ had presented a significant increase 6 h after venom injection, suggesting an increment in the resistive properties, mainly in airways, with possible hypersensitivity reactions and accumulation of secretion. However, a direct or indirect effect of the venom cannot be ruled out. The values of viscoelastic pressures in our study, represented for $\Delta P2$ and strengthened for ΔE, had presented a 12 h peak after inoculation of CdtV. These results can be attributed to the indirect actions of the venom, mainly to the observed inflammatory process in the histological study. The values of the total pressure variation of the lung, represented for ΔP_{tot}, demonstrate that in 24 h period, an increase of the total pressure for displacement of flow in the lung occurs, that is,

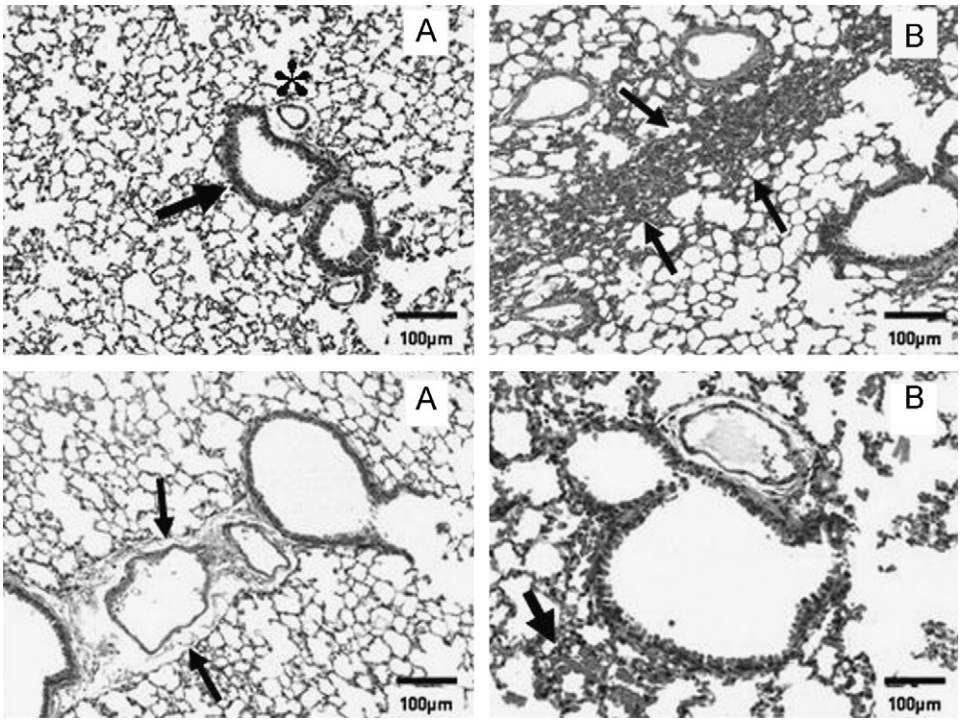

Fig. 4. Representative photo-micrographs of lung histology stained with H/E. In (A) control group, it shows the morphological appearance of lung tissue, with normal airways (arrows) and blood vessel (star), (B) 3 h group, show an increase in inflammatory cells (arrow), (C) 6 h group, show edema in parenchyma (arrows), and (D) 12 h group show diffuse hemorrhage (arrow). Scale bar = 100 μm.

greater pressure must be generated to win the elastic forces. We observe that this parameter returns to control values in the end of the 24 h showing a progressive decrease of lung mechanics properties in function of the time. Some experimental models indicate that after local tissue injury caused by venoms, several processes can be activated, such as adjusted muscular regeneration, wound repair and the restoration the injured tissue without functional regeneration (Cardoso et al., 2003; Andrade et al., 2004). The results found in our study show that CdtV provokes mechanical alterations in the pulmonary tissue that returns to normal function after 24 h. A possible explanation for these results could be the fact that the administration route used for our experimental model was intramuscular (i.m.), it is possible that the amount of venom that arrived in the lung was not enough to cause an irreversible damage to the lung tissue. Therefore, the changes observed in our study could be due to the release of inflammatory mediators induced by the venom (Cruz et al., 2005). The restoration of mechanical properties to basal parameters after 24 h cannot be attributed to regeneration due to lack of evidences. In addition, in our study we do not use the crotalic antivenom because the literature (Jurkovich et al., 1988; Brooks et al., 2002) shows that patients developed hypersensitivity reactions, manifested as respiratory compromise, while receiving anticrotalic venom. Therefore, studies are needed to investigate the respiratory compromise after antivenom administration.

CdtV is constituted of enzymes, toxins and peptides (Varanda and Giannini, 1994). Crotoxin is the most important of them, it is a heterodimeric protein composed of a basic Asp49-PLA_2 and an acidic protein named crotapotin (Breithaupt et al., 1974). The presence of hemorrhage 12 h after the venom injection is contradictory to literature, since CdtV possesses high neurotoxic activity and scarce proteolitic, hemorrhagic and edematogenic activity (Santoro et al., 1999), however, the presence of the associated crotoxin to a high PLA_2 activity, makes the CdtV miotoxic, being able to cause the hemorrhagic found in this study. Gutiérrez and Ownby (2003) showed that after distribution in the body, PLA_2 has systemic action, it binds in the receptor of high affinity in the muscular membrane

proteins, inducing the systemic myotoxicity. It is therefore possible that crotoxin plays a role in the venom-induced hemorrhage, a hypothesis that needs to be addressed in future studies.

The initial phase of pulmonary acute injury is characterized by the increase in the endothelial and epithelial permeability, followed of a fibroproliferative phase that can result in a repair of the injured lung or in a gradual obliteration of the interstitial and alveolar compartments (Meduri et al., 1995). We found perivascular edema, 6 h after CdtV injection (not quantified in our study) that can be related to the indirect factors generated by CdtV and attributed to a systemic action of PLA_2. Also, Santoro et al. (1999) demonstrated an edematogenic effect induced by CdtV, in paw tissue, that was not dose-dependent, and the maximum edema formation was observed 30 min after the venom injection and it did not reach values higher than 35%.

Mionecrose induced by PLA_2 is followed by the infiltration of inflammatory cells to the affected muscle. Polymorphnuclear leukocytes, mainly neutrophils, are the first ones to arrive, with 3–6 h and the macrophages predominate in delayed intervals of time (Harris and Maltin, 1982). In our study, histological analysis demonstrated an increase in polymorphonuclear cells, with a peak between 3 and 6 h, suggesting a direct action of the PLA_2 on the pulmonary tissue. Neutrophils possess an important role in the mediation of the microvascular damages and also contribute for the pulmonary tissue injuries (Macnee and Selby, 1993). Leukocytes play a central role in the inflammatory process owing to their capacity to produce a variety of inflammatory mediators (Zheng et al., 1999). It was found, in patients bitten by CdtV, an increase of IL-6 and IL-8 in serum (Cardoso et al., 2003), it is possible that this mediators may induce neutrophil activation, contributing to the formation of a cellular infiltrate in the lung.

In conclusion, the present study provides evidences that *Crotalus durissus terrificus* venom provokes mechanical alterations in the pulmonary tissue, which could be associated with histopathological alterations, features that returned to normal function after 24 h.

Acknowledgments

This work was supported by FAPESP and FVE-UNIVAP and Paula Naomi Nonaka is a fellowship of CAPES.

References

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.

Amaral, C.F.S., Magalhães, R.A., Rezende, N.A., 1991. Comprometimento respiratório secundário a acidente ofídico crotálico. Rev. Inst. Med. Trop. São Paulo 33, 251–255.

Bancher, W., Rosa, R.R., Furlanetto, R.S., 1973. Estudos sobre a fixação eletiva e quantitativa do veneno de *Crotalus durissus terrificus* nos tecidos nervoso, renal, hepático e muscular de *mus-musculus* Linnaeus. Mem. Inst. Butantan 37, 139.

Barraviera, B., 1990. Acidentes por animais peçonhentos. Arquivos Brasil. Med. 64, 14–20.

Barraviera, B., 1993. Estudo clínico dos acidentes ofídicos. J. Brasil. Med. 65, 209–250.

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.

Bates, J.H.T., Ludwig, M.S., Sly, P.D., Brown, K., Martin, J.G., Fredberg, J.J., 1988. Interrupter resistance elucidated by alveolar measurement in open-chest normal dogs. J. Appl. Physiol. 65, 408–414.

Breithaupt, H., Rübsamen, K., Habermann, E., 1974. Biochemistry and pharmacology of the crotoxin complex. Biochemical analysis of crotapotin and the basic phospholipase A. Eur. J. Biochem. 49, 333–345.

Brooks, D.E., Graeme, K.A., Ruha, A.M., Tanken, D.A., 2002. Respiratory compromise in patients with rattlesnake envenomation. J. Emerg. Med. 23, 329–332.

Cardoso, J.L.C., França, F.O.S., Wen, F.H., Málaque, C.M.S., Haddad Jr., V., 2003. Animais Peçonhentos no Brasil: Biología, Clínica e Terapêutica dos Acidentes. Sarvier, São Paulo.

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

Cruz, A.H., Mendonça, R.Z., Petricevich, V.L., 2005. *Crotalus durissus terrificus* venom interferes with morphological, functional, and biochemical changes in murine macrophage. Mediat. Inflamm. 14, 349–359.

Gutiérrez, J.M., Ownby, C.L., 2003. Skeletal muscle degeneration induced by venoms phospholipases A_2: insights into the mechanisms of local and systemic myotoxicity. Toxicon 42, 915–931.

Harris, J.B., Maltin, C.A., 1982. Myotoxic activity of the crude venoms and the principal neurotoxin, taipoxin, of Australian taipan, *Oxyuranus scutellatus*. Br. J. Pharmacol. 76, 61–75.

Jorge, M.T., Ribeiro, L.A., 1992. The epidemiology and clinical picture of an accidental bite by the South American rattlesnake (*Crotalus durissus terrificus*). Rev. Inst. Med. Trop. Sao Paulo 34 (4), 347–354.

Jurkovich, G.J., Luterman, A., McCullar, K., Ramenofsky, M.L., Curreri, P.W., 1988. Complications of Crotalidae antivenin therapy. J Trauma 28, 1032–1037.

Lanças, T., Kasahara, D.I., Prado, C.M., Tibério, I.F., Martins, M.A., Dolhnikoff, M., 2006. Comparison of early and late responses to antigen of sensitized guinea pigs parenchymal lung strips. J. Appl. Physiol. 100, 1610–1616.

Macnee, W., Selby, C., 1993. New perspectives on basic mechanisms in lung disease. 2. Neutrophil traffic in the lungs: role of haemodynamics, cell adhesion, and deformability. Thorax 48, 79–88.

Meduri, G.U., Eltorky, M., Winer-Muram, H.T., 1995. The fibroproliferative phase of late adult respiratory distress syndrome. Sem. Respir. Infect. 10 (3), 154–175.

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

Oguiura, N., Boni-Mitake, M., Rádis-Baptista, G., 2005. New view on crotamine, a small basic polypeptide myotoxin from South American rattlesnake venom. Toxicon 46, 363–370.

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.

Santoro, M.L., Silva, M.C.C.S., Gonçalves, L.R.C., Santos, S.M.A., Cardoso, D.F., Ferreira, I.L.L., Saiki, M., Peres, C.A., Martins, I.S.S., 1999. Comparison of the biological activities in venoms from three subspecies of the South American rattlesnake (*Crotalus durissus terrificus, C. durrissus cascavella* and *C. durrisus collilineatus*). Comp. Biochem. Physiol. 122, 61–73.

Santos, R.M.M., Oliveira, L.C., Estevão-Costa, M.I., Lima, M.E., Santoro, M.M., Fortes-Dias, C., 2005. Inhibition of crotoxin binding to synaptosomes by a receptor-like protein from *Crotalus durissus terrificus* (the South American rattlesnake). Biochim. Biophys. Acta 1717, 27–33.

Santos, F.B., Nagato, L.K.S., Boechem, N.M., Negri, E.M., Guimarães, A., Capelozzi, V.L., Faffe, D.S., Zin, W.A., Rocco, P.R.M., 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.

Toyama, M.H., Oliveira, D.G., Beriam, L.O.S., Novello, J.C., Rodrigeus-Simioni, L., Marangoni, S., 2003. Structural, enzimatic and biological properties of new PLA_2 isoform from *Crotalus durissus terrificus* venom. Toxicon 41, 1033–1038.

Varanda, E.A., Giannini, M.J.S.M., 1994. Bioquímica de venenos de serpentes. In: Barraviera, B. (Ed.), Venenos animais: uma visão integrada. EPUC, Rio de Janeiro, pp. 205–224.

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

Vital Brazil, O., 1972. Neurotoxins from the South American rattlesnake venom. Journal of the Formosan Medical Association. 71, 394–400. In: Damico, D.C.S., Bueno, L.G.F., Rodrigues-Simioni, L., Marangoni, S., Cruz-Höfling, M.A., Novello, J.C. (Eds.), Neurotoxic and myotoxic actions from *Lachesis muta muta* (surucucu) whole venom on the mouse and chick nerve-muscle preparations. Toxicon 46, 222–229.

Weibel, E.R., 1990. Morphometry: stereological theory and practical methods. In: Gil, J. (Ed.), Models of Lung Disease—Microscopy and Structural Methods. Marcel Dekker, New York, pp. 199–247.

Zin, W.A., Rocco, P.R.M., 1999. Movimentos Respiratórios e Mecânica Respiratória. In: Aires, M.M. (Ed.), Guanabara-Koogan, Fisiologia, pp. 503–525.

Zheng, L., Zomerdijk, T.P., Van Den Barsellar, M.T., Geertsma, M.F., Van Furth, R., Nibbering, P.H., 1999. Arachidonic acid, but not its metabolites, is essential for FcgammaR-stimulated intracellular killing of *Staphylococcus aureus* by human monocytes. Immulogy 96, 90–97.