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Effect of Vagotomy on *Mesobuthus tamulus* Venom-Induced Pulmonary Edema and Compliance in Rats.

Ratna Pandey, Phani Bhushan Singh, and Shripad B. Deshpande*.

Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India

ABSTRACT

Red scorpion (*Mesobuthus tumulus*) envenomation is a common life threatening medical problem in India. Pulmonary edema and decrease in lung compliance is a common manifestation after scorpion envenomation. In the present study, role of vagus in mediating *Mesobuthus tamulus* (MBT) venom-induced alteration in pulmonary water content and compliance was ascertained in anesthetized adult rats. The rats were divided into 3 groups. In group I, MBT venom was administered. In group II (control group), equal volume of saline was injected. In group III, MBT venom was administered in vagotomized rats. Pulmonary water content and lung compliance was determined in all groups after death of the animals or after sacrificing the rats at the end of observation period (120) min. Exposure to MBT venom produced significant increase in pulmonary water content (84 %) as compared to control rats (73 %). Lung compliance (0.14 ml/mm Hg) was decreased as compared to control group (0.21 ml/mm Hg) in envenomed animals. Compliance in vagotomized rats (0.16 ml/mm Hg) after exposure to MBT venom was not different from the venom alone group but vagotomy prevented the development of pulmonary edema. Pulmonary water content in vagotomized rats (74%) was similar to the control group (73%). The results indicate that vagus plays a vital role in producing pulmonary edema.

Keywords: Mesobuthus tamulus envenomation, Vagotomy, Pulmonary edema, Lung compliance

Corresponding author

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INTRODUCTION

Indian red scorpion, *Mesobuthus tamulus* (BT) venom produce severe changes in cardiovascular system, respiratory system and other systems [1,2,3,4,5]. Pulmonary edema due to scorpion stings has been reported earlier [6,7,8,9]. Pulmonary edema along with cardiac and other complications has been proposed to be the cause of death in human beings and experimental animals [8,10,11]. Vagus is reported to play a role in mediating inflammation and may be responsible for pulmonary edema. Pulmonary edema or congestion is a natural stimulus for J reflex as described by Paintal which causes apnea, hypotension and bradycardia [12]. The impulse for this reflex is carried by vagus. Thus, pulmonary edema and excitation of J reflex by it may cause deleterious effects on various tissues or systems. Pulmonary edema is supposed to alter the lung compliance and may further deteriorate the condition. Thus, vagus seems to play an important role in the pathogenesis of MBT venom-induced toxicity. Therefore, we hypothesized that by inhibiting the message carried by vagus, we may protect the animals against MBT venom-induced pulmonary edema and its deleterious effects. Therefore, the present study was undertaken to elucidate the role of vagus in reversing the pulmonary edema and compliance of the lungs.

MATERIALS AND METHODS

Animals, Anesthesia and Dissection

All the experiments were performed after taking approval from the ethical clearance committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Male adult rats (175-225 g) belonging to Charles Foster strain were anaesthetized with an intraperitoneal (i.p.) injection of urethane (1.5 gm/kg). Tracheal cannulation was done to keep the respiratory tract patent followed by jugular venous cannulation to deliver saline/venom. The animals were allowed to stabilize for at least 30 min after the dissection.

Experimental Protocol

The animals were divided into 3 groups. In group I, MBT venom (5 mg/kg) was administered after the stabilization of animals. In group II, equal volume of saline was administered through the jugular vein. This group served as control. In group III, after stabilization, bilateral vagotomy was performed and 30 min after this, MBT venom (5 mg/kg) was administered. The animals were observed for 120 min or till death. In each group, lung compliance and pulmonary water content was determined after death of the animal or after sacrificing the animal with overdose of anesthesia at the end of observation period.

Determination of Compliance and Pulmonary water content

The lung compliance was determined after death of the animal or after sacrificing the animal with overdose of anesthesia. For this, 1-5 ml of air was introduced in the lungs via the tracheal cannula and corresponding change in pressure was recorded with a mercury manometer. The graph of change in pressure v/s change in volume was plotted and the slope in the linear segment was computed. The slope was taken as the compliance of the lungs. For determination of pulmonary water content, the lungs were dissected out and weighed. The lung tissue was dried in an electric oven to a constant weight for 48-72 h and then the % of pulmonary water was determined as described earlier [13].

Drugs and solutions

Crude MBT venom was obtained from the Haffkine Institute Mumbai, India. The stock solution (5 mg/ml) of MBT venom was prepared in distilled water and refrigerated. Urethane was obtained from Merck, Germany and dissolved in 0.9% saline. To anesthetize the animals, urethane was used intraperitoneally with a dose of 1.3-1.5 g/Kg.

Statistical analysis

All the data were presented as mean ± SEM. The statistical significance was determined by using Student's t-test and mentioned at appropriate places. A p value <0.05 was considered significant.

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RESULTS

Lung compliance and pulmonary water content in control rats

The compliance (ΔV/ΔP) in saline treated rats was 0.21 ml/mm Hg and the pulmonary water content was 73.4% ± 0.82 of wet lung tissue (Figure 1, 2). The animals in this group survived throughout the experimentation.

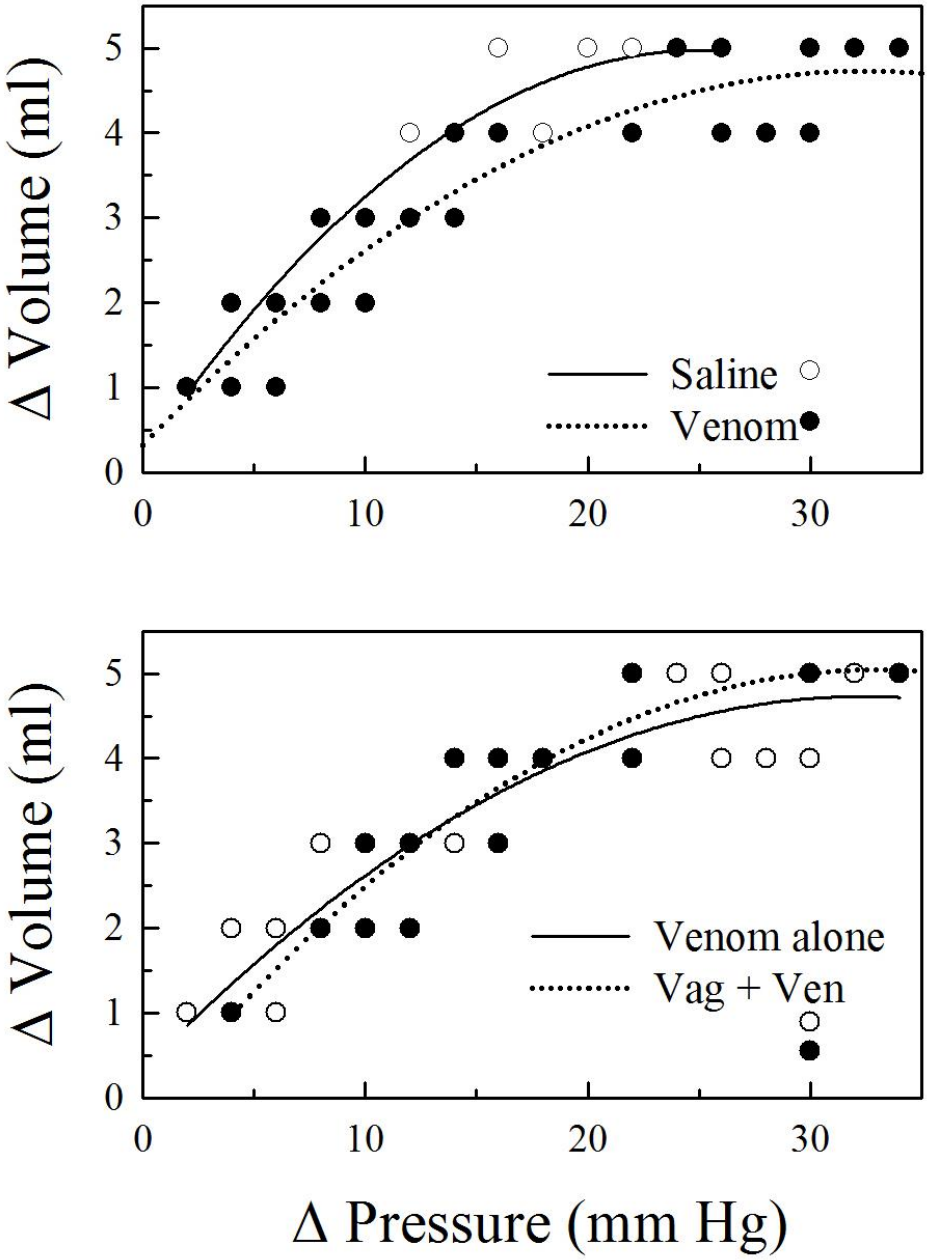

Fig 1: Scattergram showing the pressure-volume realationship of lungs in different conditions. The lines were fit to the second order of regression using sigma plot. Control data was obtained from 30 points and venom/ vag + ven data was obtained from 35 points. Many symbols/data points are overlapping. The slope of the curves show compliance (ΔV/ΔP) of the lung (0.21 ml/mm Hg for saline, 0.14 ml/mm Hg for venom only and 0.16 ml/mm Hg for Vag + ven (vagotomy + venom) group.

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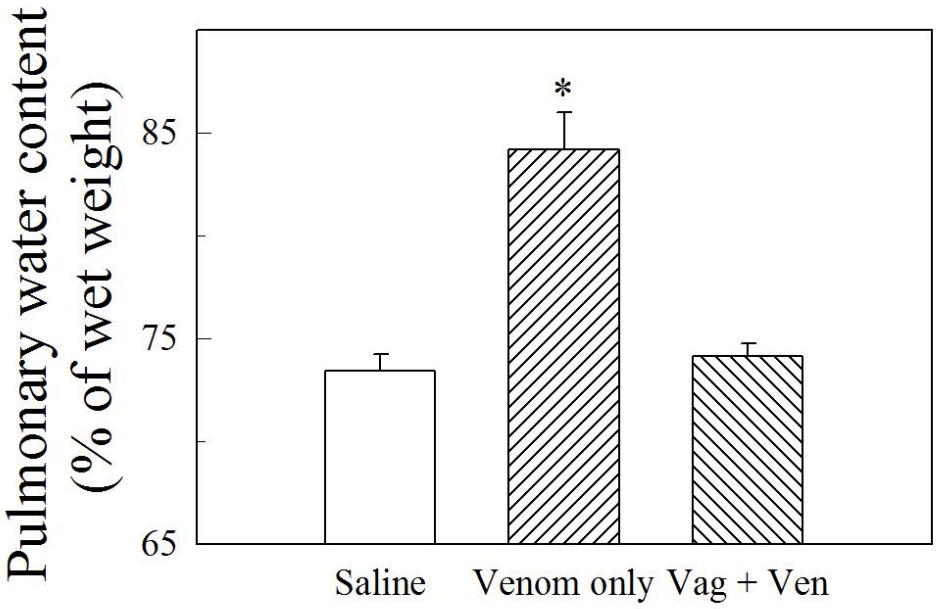

Fig 2: Histogram showing the pulmonary water content (as % of wet lung tissue) in different conditions. The values are expressed as mean ± SEM. The asterisk (*) indicates significant difference from saline and vag + ven (vagotomy + venom) group.

MBT venom decreased the lung compliance and increased pulmonary water content

The compliance (ΔV/ΔP) after MBT venom administration (0.14 ml/mm of Hg) was significantly lower than the control group (0.21 ml/mm Hg; Figure 1). The pulmonary water content in venom treated rats was 84 ± 1.8% which was significantly greater than control group (73.4 ± 0.82; Figure 2, $p < 0.05$, Student's t-test for unpaired observations). All the animals in this group died within 60 min after venom administration.

Bilateral vagotomy protects against pulmonary edema

The compliance (ΔV/ΔP) in vagotomized rats after MBT venom exposure (0.16 ml/mm Hg) was similar to venom only group (0.14 ml/mm Hg) and was significantly lower as compared to control group (0.21 ml/mmHg; Figure 1). The increase in pulmonary water content as observed in venom only group (84.2 ± 1.8) was not seen in bilaterally vagotomized rats after MBT venom exposure (74.16 ± 0.6). The pulmonary water content in this group was similar to control group (73.4 ± 0.82; Figure 2, $p > 0.05$, Student's t test for unpaired observations). Out of 6 animals in this group, 4 survived throughout the period of observation (120 min) and 2 died by 75 min. The overall survival in this group of animals was more as compared to venom only group (< 60 min).

DISCUSSION

MBT venom produced pulmonary edema and decreased lung compliance in rats in this study also as reported earlier [9]. However, administration of MBT venom in bilaterally vagotomized rats failed to produce pulmonary edema but the lung compliance in these rats was similar to that observed in venom only group.

The typical scorpion envenomation syndrome is characterized by alteration in cardiopulmonary parameters and the production of pulmonary edema [9,13,14]. Our data provide evidence for the involvement of vagus in producing pulmonary edema and thereby affecting overall survival of the animal.

The absence of pulmonary edema in vagotomized rats is difficult to interpret but indicate the involvement of afferent or efferent vagal fibers in producing pulmonary edema. Earlier study reported that vagal afferent discharges were doubled after scorpion envenomation [5]. Thus there is increased afferent volley through the vagus in envenomed animals because of pulmonary edema or other causes. Further,

Pulmonary congestion/edema are the natural stimulants to evoke visceral reflexes including J-reflex which is mediated through the vagus nerve [15]. These visceral reflexes stimulate the high threshold cardio-pulmonary receptors to produce apnea, hypotension and bradycardia. This is the classical triad described for J-reflex [12]. In a study elsewhere, ondansetron blocked the J receptor activation and venom-induced increase in vagal afferent discharge but failed to prevent pulmonary edema formation [5]. These observations suggest the non-involvement of vagal afferents in producing pulmonary edema. The bronchial smooth muscles receive cholinergic vagal efferents. The stimulation of the vagal efferents produces bronchospasm. Further, MBT venom is shown to increase the excitability of peripheral axons [4, 16) and such excitation of vagal efferents further enhance the bronchospasm. The mechanism for the absence of edema in the vagotomized rats can be explained on the basis of bronchospasm. Brochospasm induces hypoxia leading to pulmonary hypertension and development of pulmonary edema. After vagotomy, the bronchial smooth muscles are dennervated and no spasm is produced. Absence of pulmonary edema after venom administration in vagotomized rats in our study supports this hypothesis. Pulmonary edema affects the gaseous exchange and thus produces hypoxia. In addition pulmonary edema is known to decrease the lung compliance. Decreased lung compliance will further aggravate the condition by affecting the ventilation. Survival of 4/6 vagotomized rats after venom exposure and prolonged survival in rest of 2 rats may be due to absence of pulmonary edema. Thus, our observations indicate that pulmonary edema is an important factor affecting the overall survival after MBT envenomation. Other mechanisms may be implicated for the death of 2/6 rats.

Further, our study show that in vagotomized rats the lung compliance was similar to venom only group indicating that mechanism other than vagus is involved in decreasing the lung compliance after venom exposure.

CONCLUSION

In conclusion, our study demonstrates the pulmonary edema in scorpion envenomation is mediated through vagus. Bilateral vagotomy prevented MBT venom-induced pulmonary edema but failed to improve lung compliance.

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