National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Role of vagus in mediating the toxicity induced by *Mesobuthus tamulus* venom in rats

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Received: April 04, 2017; **Accepted:** April 17, 2017

ABSTRACT

Background: *Mesobuthus tumulus* (red scorpion, MBT) envenomation is a serious health problem in tropical countries and is responsible for high morbidity and mortality. **Aims and Objectives:** Earlier reports about the role of vagus in producing MBT venom-induced toxicity are conflicting. Therefore, in this study, the role of vagus in MBT venom-induced toxicity in rats was evaluated. **Materials and Methods:** The rats were divided into three groups. In group I, only saline was injected. This group served as control. In group II, MBT venom was injected. In group III, MBT venom was injected in vagotomized rats. Mean arterial pressure (MAP), ECG (for heart rate [HR]), respiratory rate, pulmonary water content, and survival time were determined in all groups. **Results:** Exposure to MBT venom in rats produced prolonged apnea with intermittent shallow breathing and was accompanied by an instantaneous decrease, followed by an increase and then a progressive decrease in MAP and HR leading to death within 60 min. There was increased pulmonary water content (82% vs. 74% in controls). MBT venom in vagotomized rats produced immediate changes as before, but these changes recovered to reasonably good level within 30 min and the animals survived for >120 min. Pulmonary water content in vagotomized rats was similar to control group. **Conclusion:** The results indicate that vagus plays a vital role for the toxic effects produced by MBT venom.

KEY WORDS: Vagotomy; Pulmonary Edema; *Mesobuthus Tumulus* Envenomation; Apnea

INTRODUCTION

Indian red scorpion, *Mesobuthus tamulus* (MBT) venom produces severe changes in the central nervous system, autonomic nervous system, cardiovascular system, respiratory system, and other systems.[1-6] Reports from this laboratory have shown that the animals died within 60 min of exposure to BT venom.[3] The cause of death was attributed to the respiratory failure and respiratory arrest.[3] In studies elsewhere, acute myocarditis, conduction blockade, cardiac arrhythmias, and pulmonary edema were also implicated for the lethal effects of venom.[1,2-6] It has been shown that BT venom increased the phenyl diguanide-sensitive vagal activity indicating the role of vagus.[4] The vagal activity although was completely blocked by ondansetron, a 5-HT_3 receptor antagonist, only aprotinin was able to prevent the pulmonary edema formation and the increased vagal activity.[5] Apnea is also produced by the activation of vasosensory reflexes by algogenic substances.[7] Since venom contains and/or releases several algogenic substances, it may be possible that these agents activate the vasosensory reflexes and produce apnea.[7] The afferents for vasosensory reflexes may also be carried by the vagus. Thus, vagus may play an important role in the pathogenesis of BT venom toxicity, but the role of vagus is not clearly known. Therefore, the present study was undertaken to elucidate the role of vagus in red scorpion MBT venom-induced toxicity.

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0410217042017	

MATERIALS AND METHODS

Animals, Anesthesia, and Dissection

The animal experiments were performed after obtaining 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 injection of urethane (1.5 g/kg). Tracheal cannulation was done to keep the respiratory tract patent followed by jugular venous cannulation to deliver saline/venom. Femoral artery cannulation was carried out to record blood pressure (BP). The animals were allowed to stabilize for at least 30 min after the surgical procedures.

Recording of Cardiopulmonary Parameters

For recording respiratory movements, the skin over xiphisternum was secured with a thread and connected to a force displacement transducer. The respiratory movements were recorded by a chart recorder through a bridge amplifier. For recording of BP, femoral artery was cleared from the surrounding tissue and cannulated. The cannula was connected to a pressure transducer (Statham transducer). The BP was recorded by connecting the pressure transducer to a bridge amplifier and through a galvanometer the deflections were recorded using a chart recorder. Mean arterial pressure (MAP) was determined from the calibrations using a mercury manometer. The electrocardiographic potentials (ECG) were recorded with the help of needle electrodes using standard limb lead II configuration. Heart rate (HR) was calculated manually from R-R interval of ECG.

Experimental Protocol

The animals were divided into three groups (n = 6 in each group).

Group I (saline/control group)

After stabilization and initial recordings of respiratory rate (RR), BP, and ECG (for HR), equal volume of saline was administered through the jugular vein and recordings were taken for 5 min initially and then intermittently at 15 min intervals for 2 h. This group served as control.

Group II (venom-only group)

After initial recordings of RR, BP, and ECG, MBT venom (5mg/kg) was administered and subsequent recordings were taken for 5 min initially and then intermittently at 15 min intervals for 120 min or till the death of rats. We have chosen the dose of venom (5mg/kg) as reported in our earlier study that produced severe alteration in the cardiopulmonary parameters leading to death of the animals.[8]

Group III (vagotomy+venom group)

After initial recordings of RR, BP, and ECG, bilateral vagotomy was performed, and 30 min after this, MBT venom (5 mg/kg) was administered. Subsequent responses were recorded as mentioned in Group II.

Determination of pulmonary water content

For determination of pulmonary water, the lungs were dissected out and weighed at the end of experiment. The lung tissue was dried in an electric oven to a constant weight for 48-72 h and then the percentage of pulmonary water was determined as described earlier.[4]

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 Sigma-Aldrich Inc, St. Louis, USA.

Statistical Analysis

All the data were presented as mean±SEM (n = 6 in each group). The statistical significance was determined using two-way ANOVA and Student's t-test and mentioned at appropriate places. A $P < 0.05$ was considered statistically significant.

RESULTS

Cardiopulmonary Parameters in Control Rats

The resting MAP, HR, and RR in saline-treated (control) group were 66 ± 4 mmHg, 284 ± 2 beats/min and 86 ± 8/min, respectively. Slight initial changes in MAP, HR, and RR were observed after the injection of equal volume of saline, but they remained at the same level during the entire period of observation (120 min). Pulmonary water content was 74.2% of wet lung tissue in these rats. The animals in this group survived throughout the period of observation (120 min).

MBT Venom Altered the Various Cardiopulmonary Parameters

There was instantaneous fall in MAP after MBT venom (5 mg/kg). The fall was about 18% followed by a rise in pressure over the initial level. However, after 15 min, the MAP dropped progressively (Figure 1). There was marked decrease in the HR (about 50%) which never returned to initial value (Figure 2). There was a drastic reduction in RR after MBT venom. In five of seven preparations, immediately after the injection of venom, a long-lasting apnea was observed. Apnea was followed by a gasping type of respiration (Figure 3) and finally death within 60 min. The pulmonary water content in venom treated rats was 82 ± 2.1% which

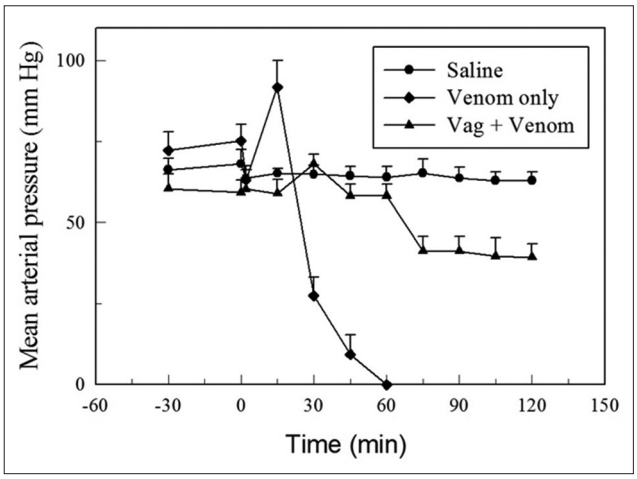

Figure 1: Effect of *Mesobuthus tumulus* venom on mean arterial pressure in different groups. Venom was administered at 0 time. The changes in vagotomized rats are significantly different from venom-only group (P<0.05, two-way ANOVA)

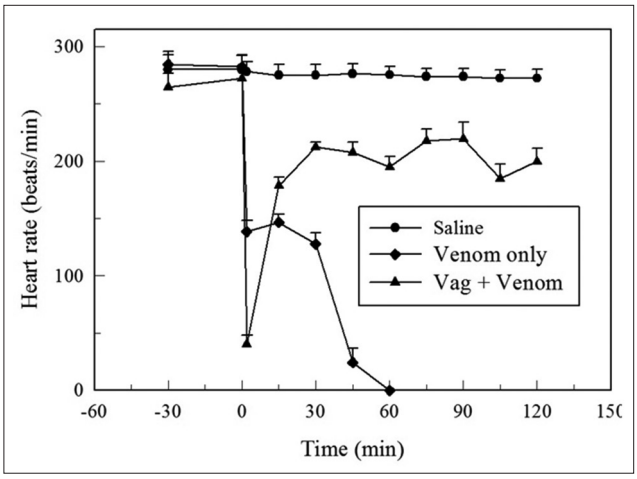

Figure 2: Effect of *Mesobuthus tumulus* venom on heart rate in different groups. Venom was administered at 0 time. The changes in vagotomized rats are significantly different from venom-only group (P<0.05, two-way ANOVA)

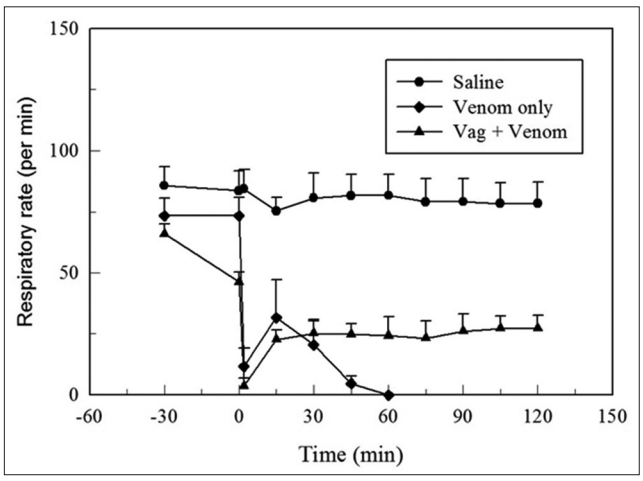

Figure 3: Effect of *Mesobuthus tumulus* venom on respiratory rate in different groups. Venom was administered at 0 time. The changes in vagotomized rats are significantly different from the venom-only group (P<0.05, two-way ANOVA)

was significantly greater than control rats (74 ± 2, Table 1, $P < 0.05$ Student's t-test for unpaired observations).

Bilateral Vagotomy Protects Against the Toxic Effects of MBT Venom

The MAP before vagotomy was 61 ± 5 mmHg and the MAP after vagotomy was slightly increased but not significantly. Administration of MBT venom in these animals did not produce any significant change in MAP up to 60 min. The typical pattern of fall then rise and subsequently progressive fall in MAP was also not observed after MBT venom in vagotomized rats. However, after 60 min, there was a clear-cut fall in MAP of about 20 mmHg which remained at the same level throughout the period of observation (Figure 1).

The HR before vagotomy was 265 ± 25 beats/min and after bilateral vagotomy slight augmentation in HR was seen. Injection of MBT venom in these rats produced immediate profound decrease in HR; however, within 15 min, the rate was restored to 70-80% of the initial level, and this rate was maintained with slight variation till the end of experimentation (Figure 2).

The RR before vagotomy was around 65/min and the rate after vagotomy was decreased significantly (about 40% of the initial value). There was marked decrease in RR after MBT venom in vagotomized rats as also seen in case of venom treated rats. However, within 15 min, there was about 70% recovery and the rate was maintained at this level throughout the recording period (Figure 3).

The pulmonary water content in bilaterally vagotomized rats after MBT venom exposure (72 ± 3.2%) was significantly lesser than venom only group (82 ± 2.1%) and was similar to control group (74 ± 2%, Table 1).

DISCUSSION

In this study, the MBT venom produced typical envenomation syndrome characterized by increased autonomic activity, alteration in cardiopulmonary parameters, and production of pulmonary edema.[4,9,10] Our data clearly demonstrate the instantaneous prolonged apnea followed by cardiac abnormalities after MBT venom. Further, our results provide evidence for the involvement of vagus in mediating the toxicity and overall survival of the animal.

Table 1: Pulmonary water content in different groups

Experimental conditions	Pulmonary water content (% of wet weight)
Saline-treated (control) group	74±2.0
Venom-only group	82±2.1*
Vagotomy + venom group	72±6.2

*: $p < 0.05$ (Students t test for unpaired observations) as compared to saline treated group and Vagotomy + venom group

The scorpion envenomation produced immediate transient hypotension, followed by hypertension and subsequently a progressive fall in MAP. In other studies also, MBT venom produced changes in MAP.[11,12] However, the simultaneous effect on the HR and respiratory parameters was not available in the above studies.

Pulmonary congestion/edema is the natural stimulant to evoke visceral reflexes.[13] These visceral reflexes are also evoked by chemicals such as kinin, histamine, prostaglandin, and serotonin, which stimulate the high threshold cardiopulmonary receptors to produce apnea, hypotension, and bradycardia. This is the classical triad described for J-reflex or Bezold–Jarisch reflex.[14,15] Responses of J-reflex stimulation was observed in our study also. The afferents of these reflexes are carried through the vagus. Our results after vagotomy attenuated the maximal apnea time significantly but did not abolish the apnea. The RR returned to the initial level after 5-10 min in vagotomized rats treated with venom. These observations uncover two aspects: First, the existence of mechanism other than those mediated by vagus for the production of apnea; second, vagus carries important vital information which reflexly aggravates the pathogenesis of envenomation.

The vagotomized rats though manifested with sign of envenomation survived throughout the period of observation. In the present observation, the pulmonary edema was a consistent feature after exposure to venom. Further, vagotomy prevented the pulmonary edema formation (Table 1). The mechanism for the absence of edema in the vagotomized rats is difficult to interpret. The data with aprotinin elsewhere fully support for the elevation of endogenous kinins by BT venom.[5,6] In addition to kinin, PGs are also implicated in venom-induced changes.[6,16] Besides these inflammatory mediators, venom contains histamine, serotonin, and other peptides which produce pain. There is possibility that all these chemical mediators stimulate or excite the vagal receptors or afferents to induce the inhibitory effect on the respiratory center resulting in respiratory failure and death of the animal. Thus, this study indicates the involvement of vagus in MBT envenomation. The changes in the cardiovascular parameters (MAP and HR) may be secondary to changes in the respiratory parameters as these two systems are interdependent.

CONCLUSIONS

Our study demonstrates that vagus plays an important role in mediating the MBT venom-induced toxicity.

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How to cite this article: Pandey R, Tiwari AK, Deshpande SB. Role of vagus in mediating the toxicity induced by *Mesobuthus tamulus* venom in rats. Natl J Physiol Pharm Pharmacol 2017;7(8):835-838.

Source of Support: Nil, **Conflict of Interest:** None declared.