Morphine blocks the *Mesobuthus tamulus* venom-induced augmentation of phenyldiguanide reflex and pulmonary edema in anesthetized rats

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**Introduction**

Cardiogenic pulmonary edema is a common characteristic feature seen in scorpion envenomation syndrome. It is attributed to acute myocarditis, left ventricular failure and hemodynamic alterations produced by scorpion envenomation. In addition, some noncardiogenic factors are also proposed for pulmonary edema after scorpion envenomation. They include inflammatory mediators, pulmonary damage, surfactant deficiency and toxin present in *Mesobuthus tamulus* (MBT) venom. Scorpion venom-induced pulmonary edema has been shown to augment the bradycardiac reflexes elicited by phenyldiguanide (PDG), phenylbiguanide (PBG), and capsaicin. The augmentation of PDG/PBG-induced...
bradycardia after envenomation has been explained on the basis of increased excitability of juxta-pulmonary capillary receptors (J receptors) due to pulmonary edema and sensitization of the vagal afferents.\textsuperscript{14,15} Further, it has been shown that blockade of pulmonary edema by aprotinin (kinin synthase inhibitor) or N-nitro L-arginine methyl ester (nitric oxide synthase inhibitor) prevents the augmentation of PDG-induced reflexes.\textsuperscript{[5]}

Acute pulmonary edema is an emergency condition after envenomation.\textsuperscript{[11–13]} Several treatment strategies have been advocated in the treatment of acute pulmonary edema produced by scorpion envenomation.\textsuperscript{[11,12]} Even though morphine is used in the treatment of cardiogenic pulmonary edema, its use in scorpion envenomation is not established.\textsuperscript{[6]} Therefore, the present study was undertaken to evaluate the effect of morphine on MBT venom-induced toxicity. MBT venom-induced responses (augmentation of PDG reflex and increased pulmonary water content) were taken as indicators of venom-induced toxicity.

Materials and Methods

Animals, Anesthesia, and Recording Procedure

The animal experiments were performed after obtaining approval from the Institutional Ethical Clearance Committee (Dean/13-14/CAEC/190). Adult female rats of Charles-Foster strain (150–225 g, 4–6 months old) were used in this study. The animals were housed in a temperature, humidity, and light (12 h: 12 h light dark period) controlled room. Two to three animals were placed in each plastic cage and provided with ad libitum food and water. The animals were anesthetized with urethane (1.5 g/kg body weight; intraperitoneally).

Trachea and jugular vein were cannulated as reported earlier.\textsuperscript{[7,9]} Tracheal cannulation was used to keep the respiratory tract patent and jugular venous cannulation for drug administration. Eletrocardiographic potentials were recorded by connecting the needle electrodes in standard limb lead-II configuration.

Drugs and Solutions

Lyophilized MBT venom was procured from Haffkine Institute, Mumbai, India. Morphine was procured from Government Opium and alkaloid works, Ghazipur (U.P), India. A single dose of morphine was administered intravenously (IV) to each animal to ensure the maximum potency of the drug. A stock solution of MBT venom (1 mg/ml) was prepared in distilled water. The required dilutions were prepared in normal saline at the time of administration. The volumes of injections were kept at 0.1 ml.

Experimental Protocol

The animals were stabilized for 30 min before subjecting to the experimental procedure. The animals were divided into three groups.

In group I (control; \( n = 5 \)), PDG (10 µg/kg) reflex response was obtained initially, 10 min after saline (0.1 ml) administration and 30 min after saline (0.1 ml) again in the same animal. This group served as time-matched control group.

In group II (MBT venom only; \( n = 6 \)), PDG (10 µg/kg) reflex response was obtained initially, 10 min after saline (0.1 ml) and 30 min after MBT venom (100 µg/kg) administration in the same animal.

In group III (morphine + MBT venom; \( n = 5 \)), PDG (10 µg/kg) reflex response was obtained initially, 10 min after morphine (1 mg/kg, IV) pretreatment and 30 min after MBT venom (100 µg/kg) administration in the same animal.

Determination of Pulmonary Water Content

The pulmonary water content was determined by physical method as described earlier.\textsuperscript{[7,9]} Briefly, at the end of each experiment both the lungs were excised, weighed and dried to a constant weight in an electric oven (at 90°C for 48 h). The difference between wet weight and dry weight was calculated to determine the water content.

Analysis of Data

Time-response area of heart rate (HR) after PDG at every 5 s up to 60 s was computed as reported earlier.\textsuperscript{[9]} In Figure 1, the computation of time-response area of PDG reflex before or after MBT venom is shown. The PDG response after saline, morphine or MBT venom was calculated in the same manner expressed as % of initial PDG time-response area. The pooled data were presented as mean ± standard error of the mean the two-way analysis of variance (ANOVA) was used to test the differences between the saline/morphine-treated groups with that of the MBT venom-only group. The multiple comparisons were made using Newman–Keul’s test. Student’s \( t \)-test for paired/unpaired observations was used as and when required. \( P < 0.05 \) was considered significant.

Figure 1: The computation of time-response area of phenyl-diguanide-induced reflex response in an experiment is shown in the lower panel. The original tracings of the same experiment showing phenyl-diguanide-induced bradycardia before and after venom are shown in the top panels. Symbol ∆ indicates point of administration of phenyl-diguanide. Note: The Mesobuthus tamulus venom-induced augmentation of phenyl-diguanide (PDG)-induced reflex response.
Results

Phenyldiguanide-induced Reflex Response was not Altered in Time-matched Control Group

The initial HR in the control group was 267 ± 8.5 beats per min, and it remained similar after 10 and 30 min of saline. PDG produced maximal bradycardiac response around 20 s and returned to initial level by 60 s. The PDG responses obtained 10 and 30 min after saline administration were similar to the initial responses [Figure 2].

Mesobuthus tamulus Venom Augmented Phenyldiguanide-induced Reflex Response

The initial HR in this group was 271 ± 25 beats per min. The basal HR after saline administration was 275 ± 21 and after MBT venom administration it was 297 ± 24 beats per min. The basal HR was not different from the initial value. PDG (10 µg/kg) produced a bradycardiac response as shown earlier [Figure 2]. The PDG reflex response obtained after saline remained similar to the initial PDG response. The PDG reflex response after MBT venom was 2.5 times the initial PDG response [Figures 1 and 2; \( P < 0.05 \), two-way ANOVA and Newman–Keul’s test].

Morphine Pretreatment Blocked Mesobuthus tamulus Venom-induced Augmentation of Phenyldiguanide Response

The initial HR value in this group was 270 ± 30 beats per min. The HR after morphine pretreatment was 216 ± 48 beats per min but was not significantly different from the initial value \( (P > 0.05) \), Student’s \( t \)-test for paired observations. The HR value after MBT venom was 114 ± 30 beats per min and was significantly less than the initial HR \( (P < 0.05) \), Student’s \( t \)-test for paired observations. In this group also, PDG induced bradycardiac response was similar to the earlier group [Figure 2]. The PDG reflex response after morphine was 66% of the initial PDG response [Figure 2]. The PDG response was not augmented after MBT venom, and it remained similar to the response after morphine [Figure 2; \( P > 0.05 \), two-way ANOVA followed by Newman–Keul’s test; \( P < 0.05 \), Student’s \( t \)-test for unpaired observations, as compared to MBT venom only group].

Mesobuthus tamulus Venom-induced Pulmonary Edema was Blocked in Morphine Pretreated Animals

The pulmonary water content in the saline control group was 78.8 ± 1.05%, and it was significantly greater in MBT venom treated group. However, the pulmonary water content in morphine pretreated animals after venom was similar to the saline control group [Figure 3].

Discussion

The results of this study demonstrate that morphine blocks MBT venom-induced pulmonary edema and the augmentation of PDG reflex response. In addition, our results confirm the augmentation of PDG reflex after MBT venom as seen in earlier reports.[5,7] Morphine is a drug of choice for treating acute pulmonary edema due to left ventricular failure.[8] Left ventricular failure is one of the cardiogenic factor implicated for scorpion venom-induced pulmonary edema.[1,2] The pulmonary edema has been shown to increase the vagal afferent volley thereby producing augmentation of vagal C-fiber reflexes.[9,10] Corresponding to earlier observations, MBT venom produced pulmonary edema and augmented the bradycardiac responses elicited by PDG.[5,7] Such augmentation was not seen in morphine pretreated animals after MBT venom [Figure 2]. Further, morphine blocked the pulmonary edema induced after scorpion envenomation [Figure 3]. Thus, it can be proposed that morphine blocks the MBT venom-induced augmentation of PDG reflex by blocking the pulmonary edema.

In our earlier reports, noncardiogenic factors have also been implicated in the development of pulmonary edema due to scorpion venom.5,7-10 In this study, morphine pretreatment blocked the pulmonary edema induced by MBT venom, and the pulmonary edema was not augmented after MBT venom, and it remained similar to the response after morphine [Figure 2; \( P > 0.05 \), two-way ANOVA followed by Newman–Keul’s test; \( P < 0.05 \), Student’s \( t \)-test for unpaired observations, as compared to MBT venom only group].

Figure 2: Mesobuthus tamulus (MBT) venom-induced augmentation of phenyldiguanide response was blocked by morphine. Pheynldiguanide responses were obtained initially, after saline/morphine and after MBT venom. The vertical bars indicate mean ± standard error of the mean values from 5 to 6 experiments as shown in Figure 1. An asterisk (*) indicates \( P < 0.05 \), two-way ANOVA and Newman–Keul’s test; @ indicates \( P < 0.05 \), Student’s \( t \)-test for paired observations as compared to the initial phenyldiguanide response area and # indicates \( P < 0.05 \), Student’s \( t \)-test for unpaired observations as compared to MBT venom only group.

Figure 3: Morphine pretreatment blocked the pulmonary edema. The pulmonary water content in control (Saline), Mesobuthus tamulus venom only (MBT) and morphine + Mesobuthus tamulus venom (Morph + MBT) groups are shown. Each bar depicts mean ± standard error of the mean values from 5 to 6 experiments in each group. An asterisk (*) indicates \( P < 0.05 \), Student’s \( t \)-test for unpaired observations as compared to the control and @, indicates \( P < 0.05 \), Student’s \( t \)-test for unpaired observations as compared to MBT venom only group.
edema after scorpion envenomation. They include pulmonary edema producing toxin, increased permeability of alveolar-capillary barrier due to the release of inflammatory mediators, pulmonary injury or surfactant deficiency. In a different set of experiments, MBT venom also augmented the capsaicin-induced reflex responses in pulmonary edema-independent manner. Capsaicin is a nociceptive agent that acts through transient receptor potential vanilloid receptor 1 (TRPV1) receptors present on the vagal afferents. Therefore, it was proposed that direct sensitization of nociceptive vagal afferents by the inflammatory mediators released after scorpion envenomation produce the reflex augmentation of capsaicin-induced responses. Morphine being an anti-nociceptive agent, it is likely that by suppressing the nociceptor activity it prevents the augmentation of the reflex response. Thus, morphine is acting in pulmonary edema-dependent or independent manners to block the augmentation of visceral reflexes elicited by PDG.

Further, morphine is a known 5-HT receptor antagonist. Hence, it can be expected that the blockade of PDG reflex is due to the anti-serotonergic action. In our earlier report, it has been shown that ondansetron, a specific 5-HT, antagonist completely blocked the PDG-induced response but not the pulmonary edema. Unlike ondansetron, morphine did not block the PDG reflex response completely [Figure 2]. However, morphine blocked the augmentation of PDG response after MBT venom administration. Hence, the effect of morphine cannot be attributed to 5-HT receptor-mediated mechanisms.

Morphine dose used in this study decreased the basal HR (though not significantly). This is suggestive for the medullary depressive action of morphine. It can be anticipated that blockade of reflex may be due to the medullary depressant action. However, it appears unlikely because the augmentation of PDG reflex and pulmonary edema were blocked after morphine pretreatment. In this study, only single dose of morphine was used. It may be necessary to find the dose that has minimal medullary depressant effects which can block the MBT venom-induced effects.

Conclusion

Taken together, our results indicate that morphine blocked the augmentation of PDG-induced reflex response by preventing the development of pulmonary edema after scorpion envenomation and its anti-nociceptive action. Hence, morphine may be considered as an alternative therapeutic strategy for pulmonary edema associated with scorpion envenomation syndrome.

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Conflicts of Interest

There are no conflicts of interest.

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