Aprotinin reverses ECG abnormalities induced by *Mesobuthus tamulus concanesis*, Pocock venom in adult rats

Ratna Pandey & Shripad B. Deshpande*

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

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The kinins are implicated in the pathogenesis of scorpion envenomation. Therefore, this study was carried out to examine the involvement of kinins for the ECG abnormalities induced by *M. tamulus concanesis*, (BT) venom in anaesthetized rats. ECG was recorded using needle electrodes with limb lead II configuration. The PR interval, QRS wave pattern, QRS duration, ST segment and heart rate were examined in saline only, venom alone, and venom after aprotinin groups. BT venom (5 mg/kg) produced heart block of varying degree and ischemia-like changes in ECG wave pattern and the animals died within 30 min after exposure to venom. In aprotinin pretreated animals, the initial ECG changes produced by venom persisted, but after 15 min the ECG pattern improved and the animals survived for the entire period of observation (120 min). The results indicate that aprotinin protected the rats against the cardiotoxicity induced by BT venom.

Keywords: Aprotinin, Arrhythmia, Cardio-toxicity, Indian red scorpion, Kinins, Myocardial ischaemia

Indian red scorpion, *Mesobuthus tamulus concanesis*, Pocock (BT) venom produces myocarditis, arrhythmia and ischaemia-like changes in ECG\(^1,2\). These abnormalities are implicated for the toxic effects of venom\(^1,2\). The venom also produces pulmonary edema in man and experimental animals\(^2,5\). The BT venom-induced pulmonary edema is shown to augment the cardiopulmonary reflexes involving J receptors\(^4,6,7\). Further, the pulmonary edema produced by venom has been shown to be due to the existence of a novel pulmonary edema producing toxin in the venom\(^5\). Aprotinin, a kinin synthesis inhibitor prevented the pulmonary edema and lethality induced by BT venom or its toxin\(^3,6,7\). All these reports indicate the pulmonary involvement for the lethality of venom. However, the toxicity produced by BT venom can not be isolated to a single organ or a system, as multi-organ failure has been proposed after envenomation\(^8,9\). Heart being a vital organ, the protective effect of aprotinin on venom- induced cardiac abnormalities thus assumes an importance. Therefore, the present study has been undertaken to elucidate the protective effects of aprotinin in reversing the ECG abnormalities induced by BT venom.

Materials and Methods

Animals, anaesthesia and dissection — The experiments were performed on healthy male adult albino rats (200-300 g) belonging to the Charles Foster strain. The animals were provided with food (Hindustan Lever Ltd.) and water *ad libitum*. They were exposed to 12:12 hr light/dark cycle. All the experiments were performed according to the guidelines of the Ethical committee of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, for conducting animal experiments.

The animals were anaesthetized with an intraperitoneal injection of urethane (1.5 g/kg body weight). Tracheal cannulation was done to keep the respiratory tract patent followed by jugular venous cannulation to deliver drugs/venom.

Drugs and solutions — Crude BT venom was obtained from the Haffkine Institute Mumbai, India. The stock solution (5 mg/ml) of BT venom was prepared in distilled water and refrigerated. Freeze dried aprotinin was obtained from Wako Pure Chemical Industries, Japan and was dissolved in distilled water. Aprotinin 6000 kallikrein inactivating unit (KIU) in 60 μl was administered intravenously as reported earlier\(^3,6,7\).

Experimental protocol — The animals were allowed to stabilize for 30 min after the surgical procedure. ECG was recorded (paper speed-50 mm/sec) with the help of needle electrodes using standard limb lead II

*Correspondent author
Phone:91-542-2369069
Fax:91-542-2367568
E-mail: desh48@yahoo.com
configuration. Animals were divided into three groups. In saline only group (time matched control; n=6), after initial ECG recording, 0.1 ml of saline was administered through jugular vein while the recordings were being taken and the recordings were continued up to 5 min. Subsequent recordings were taken at 15 min intervals up to 120 min. In venom alone group (n=6), after initial ECG recordings, 0.1 ml saline was administered and after 25 min BT venom (5 mg/kg) was administered while the recordings were being taken and the recordings were continued up to 5 min. Subsequent recordings were made at every 15 min intervals up to 120 min. In ‘aprotinin + venom’ group (n=3), after initial ECG recordings, the animals received aprotinin (6000; KIU, iv bolus) and the recordings were made after 25 min. Subsequently, BT venom (5 mg/kg) was injected while the recordings were being taken and the recordings continued up to 5 min. Further recordings were taken at every 15 min intervals up to 120 min.

Statistical analysis — The data of saline only and ‘aprotinin+venom’ groups were presented as mean±SD. In case of venom alone group, individual data are presented in Tables 1-3. The actual tracings at different times are also presented in Fig. 1. In the present experimental design, the aprotinin was considered to be protective if the aprotinin pretreatment protected the animals for 120 min (twice the survival time seen with venom alone group). Two way ANOVA was used for the comparison of data up to 15 min between various groups. Student-Newman-Keuls test was used for multiple comparisons.

Results

ECG changes in saline only group — In saline only group (n=6), the heart rate was around 260 beats/min. The mean PR interval and QRS duration were around 56 and 30 msec, respectively. These values were not altered during the entire period of observation (Tables 1-3). No abnormality in P wave, QRS wave or ST segment was observed in these rats (Fig. 1).

BT venom produced abnormalities in various ECG parameters — The ECG changes in 6 experiments after venom are presented in Fig. 1(a-f) and the corresponding data are given in Tables 1-3. Administration of BT venom resulted in flattening of P wave or heart block pattern in all animals (Fig. 1). In 4 out of 6 animals, the P wave changes persisted till the animals died (Fig. 1). In two animals, the P wave reappeared within 15 min and the PR interval was prolonged (Figs. 1c and f, Table 1).

The mean QRS duration before venom was around 30 msec. After BT venom, abnormal QRS wave pattern was seen and the QRS duration was prolonged by 2.5 times (Fig. 1, Table 2). Prominent Q wave was observed in 4 out of 6 animals (Fig. 1 a,c,e and f). In all the animals exposed to venom, there was either elevation or depression of ST segment (Fig. 1). A drastic decrease in heart rate was observed after administration of BT venom (Table 3). All the above changes persisted and the animals died by

<table>
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<th>Time (min)</th>
<th>Saline</th>
<th>Venom</th>
<th>Apr + Ven</th>
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<tr>
<td>Initial</td>
<td>56.3 ± 5.85</td>
<td>52.5</td>
<td>P-F</td>
</tr>
<tr>
<td>Saline/Apr</td>
<td>56.9 ± 6.06</td>
<td>53.2</td>
<td>P-F</td>
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<td>2</td>
<td>56.8 ± 5.75</td>
<td>53.3</td>
<td>60.0,Q-D</td>
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<td>15</td>
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<td>43.3</td>
<td>80.0</td>
</tr>
<tr>
<td>30</td>
<td>63.7 ± 13.57</td>
<td>P-F,Q-D</td>
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<tr>
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<td>57.5 ± 3.52</td>
<td>53.3</td>
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<td>75.0 ± 21.78</td>
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<td>120</td>
<td>57.5 ± 7.83</td>
<td>83.3 ± 20.81*</td>
<td>75.0 ± 22.91</td>
</tr>
</tbody>
</table>

In venom alone group, the individual values of each experiment are presented. In saline and Apr+Ven groups, mean±SD values are given. The data represent initial values after 25 min exposure to saline/aprotinin and at various time intervals after saline/venom. Death is indicated by time in parenthesis. P-F indicates flattened P wave; Q-D - distinct Q wave. *- P < 0.05 as compared to initial values (Student’s t test for unpaired observations). Venom = 5 mg/kg, Aprotinin = 6000 KIU.
Aprotinin protected against the BT venom toxicity and reversed the ECG changes — The mean PR interval before aprotinin pretreatment was around 58 msec. Aprotinin administration prolonged the PR interval by 43% (Fig. 1, Table 1). Administration of BT venom in this group (n=3) also produced transient flattening of P wave but within 15 min the P wave reappeared (Fig. 1). The PR interval returned back to aprotinin pretreated level by 30 min (Table 1). At 120 min, the PR interval returned to pre-aprotinin level (Table 1). However, all the values in this group were greater than saline only group excepting at 120 min.

In aprotinin pretreated group, the mean QRS duration before aprotinin was around 25 msec and after aprotinin it was prolonged by 56% (Fig. 1, Table 2). Administration of BT venom produced less pronounced QRS abnormalities as compared to venom alone group and QRS duration remained at aprotinin pretreated level in these rats (Fig. 1, Table 2). After 105 min, the QRS duration returned back to initial level (Table 2). However, all the values of QRS duration were greater in aprotinin pretreated group as compared to saline only group (Table 2).

Aprotinin pretreatment as such did not produce any change in ST segment (Fig. 1). Administration of BT venom in aprotinin pretreated rats produced
Immediate changes in ST segment but these changes returned to isoelectric level within 15 min and remained thereafter (Fig. 1).

Aprotinin pretreatment decreased the heart rate by 25% (Table 3). BT venom administration produced an immediate decrease in heart rate in this group also but within 15 min, the rate was restored to aprotinin pretreated level. All the animals survived throughout the period of observation as compared to venom alone group (30.7±17.4 min; \( P < 0.05 \), Student’s \( t \) test for unpaired observations).

**Discussion**

The results demonstrate that the lethal effects of BT venom are associated with myocarditis, arrhythmia, conduction blockade and ischemia-like changes in ECG. These lethal effects were not apparent in aprotinin (a kinin synthesis inhibitor) pretreated group.

The BT venom-induced ECG abnormalities manifested within 2 min of exposure to venom and remained thereafter till the animals died. Since ECG changes were not observed in saline only group, the venom-induced ECG abnormalities can not be due to the anaesthetic effect. Even though, the lethal effects produced by BT venom were not seen in aprotinin pretreated animals, the initial ECG changes seen within 2 min after the venom persisted (Fig. 1). Thus, the initial changes produced by the venom are not mediated by the kinin-dependent mechanisms. In the corresponding time period, apnea and hypotension were also reported after BT envenomation elsewhere\(^3\). Further, either in the present study or in previous study\(^3\), the animals died within 60 min after exposure to venom. These observations indicate the involvement of a single mechanism for producing ECG abnormalities as well as hypotension and respiratory changes. Looking at the time of occurrence of ECG changes in the present study and the hypotensive responses in the earlier study\(^3\), it is reasonable to propose that venom induces acute myocardial ischaemia which in turn may lead to hypotension as reported elsewhere\(^10\) to produce the lethal effects.

The BT venom-induced lethality was not observed in aprotinin pretreated rats and these animals survived with improved ECG wave pattern. The PR interval, QRS duration and HR were returned to aprotinin pretreated level. There was reappearance of P wave and reversal of ST segment changes (elevation or depression) to isoelectric line. The survival of aprotinin pretreated rats and returning of ECG parameters towards normal, when taken together, indicate the involvement of kinin mediated mechanisms for the ECG abnormalities and the lethal effects of venom. The kinins are an inflammatory mediators and are shown to be involved in BT envenomation\(^3,6,7\). In the previous study aprotinin protected the animals by preventing pulmonary oedema formation\(^3\). Similarly in other studies, aprotinin prevented the development of pulmonary oedema and the augmentation of PDG induced reflexes\(^4,7\). The present and previous findings indicate the involvement of kinins for mediating both cardiac and pulmonary abnormalities, respectively. Because of the inter-dependence of cardio-respiratory systems, the effects at one site may aggravate the toxicity at the other site.

In summary, the survival of aprotinin pretreated animals and reversal of cardiac abnormalities indicate the involvement of kinins. Therapeutic utility of kinin antagonists or kinin synthesis inhibitors in reversing the lethal effects of scorpion envenomation have to be considered.

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