Identification and characterization of an anti-platelet protein from *Daboia russelii* venom and understanding its molecular mechanism

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Chapter 6

Conclusion and future prospects

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6.1 CONCLUSIONS:

The *Daboia russelii* venom from Tanore, Rajshahi, Bangladesh exhibited in-vitro procoagulant activity, phospholipase A_2 activity, indirect haemolytic activity and inhibition of collagen induced platelet aggregation. Proteomic analysis of the venom revealed the presence of snake venom protein families responsible for the multitude of different pathophysiological manifestations observed during snakebite. The high abundance protein families present in this proteome such as PLA_2, Snaclec, SVSP, SVMP and CRISP are probably responsible for the classical symptoms observed post *Daboia russelii* envenomation such as haemostatic disturbances leading to spontaneous bleeding and painful progressive swelling. Thus, the correlation between the pathophysiological implications of *Daboia russelii* envenomation and the findings of in-vitro biochemical assays and proteomic analysis has been established.

The proteome profile showed similarities as well as differences with the previously reported proteomes of conspecific venoms from different locations of India, Pakistan, Sri Lanka and Bangladesh. The intra-specific similarities and differences indicate evolutionary relationships as well as geographical variation between *Daboia russelii* venom from different geographical locations. Such variation draws attention towards the major limitations of the present-day antivenom therapy such as paraspecific inefficacy and calls for “region-specific” antivenoms to alleviate these challenges.

The venom was screened to identify proteins with anti-platelet property and the fraction possessing maximum anti-platelet activity was subsequently studied. This fraction (P9) was found to inhibit platelet aggregation mediated by ristocetin, collagen, and thrombin. In-vitro biochemical assays revealed that P9 was anticoagulant and demonstrated PLA_2 activity. Based on mass spectrometric analysis, the fraction was identified which revealed the presence of Dabocetin and Daboxin P.

The present study evaluates the antiplatelet potential of Daboxin P as a probable natural inhibitor of thrombin from snake venom. Daboxin P inhibited aggregation of PRP as well as differentiated K-562 cells induced by thrombin. Also, it reduced thrombin-mediated calcium influx. In-silico, biochemical and biophysical interaction studies, suggests that Daboxin P inhibits thrombin-mediated aggregation of platelets by interacting with thrombin. Moreover, Daboxin P being efficient in inhibiting thrombin's role as a platelet aggregation agonist but ineffective in inhibiting the amidolytic and fibrinogenolytic activities of thrombin is an interesting observation. Therefore, Daboxin P might prove to be useful in engineering better therapeutic interventions for inhibiting thrombin mediated platelet aggregation without affecting other functions of thrombin.

6.2 FUTURE PROSPECTS:

1. Studies on interaction and synergism between Daboxin P and Dabocetin will help in the better understanding of the synergistic functions of snake venom PLA_2s and Snaclec complexes
2. Analysis of the platelet proteome will help understand any up-regulation or down-regulation of the chemical signals of the PAR signalling pathway caused due to Daboxin P
3. Designing of an anti-thrombotic peptide based on the sequence of Daboxin P that would inhibit thrombin mediated platelet aggregation without affecting other components of haemostasis