Abstract

High load of metalloproteases present in *Echis carinatus* venom are proved to be responsible for profound local tissue damage upon envenomation. Further, snake venom metalloproteases (SVMPs) have similarity in catalytic site architecture and structural domains with metzincin superfamily proteases such as MMPs, ADAMs and ADAMTS. Snake venom Kunitz-type proteins are well known to inhibit serine proteases but a few studies have also shown matrix metalloproteases (MMPs) inhibition. On the other hand, unconjugated bilirubin (UCB) is reported to inhibit ADAMTS13, a von-Willebrand factor cleaving metalloprotease. In view of the fact that MMPs, ADAMTS and SVMPs have similar catalytic site, inhibition of SVMP activity by Kunitz-type proteins and UCB remains to be studied. Recent proteomic studies of Indian elapid snake (*Bungarus caeruleus* and *Naja naja*) venoms revealed the abundance of Kunitz-type proteins. In this regard, present study aimed at inhibition of SVMPs activity of *E. carinatus* venom by UCB and also by a protease inhibitor isolated from Indian elapid snake venom. Upon screening *N. naja* venom but not *B. caeruleus* venom effectively inhibited *E. carinatus* venom-induced hemorrhage. Molecular docking studies revealed the strong binding of UCB and its dimethyl ester derivative (BD1) to the active site of modelled *E. carinatus* venom metalloprotease. Further, purification of the active principle responsible for anti-hemorrhagic effect was achieved by fractionation of *N. naja* venom in three successive chromatographic steps. SDS-PAGE revealed that purified anti-hemorrhagic protein (NNAh) has an apparent molecular mass of ~44 kDa and single peak in RP-HPLC demonstrated its homogeneity. NNAh, UCB and BD1 were effective in inhibiting the hemorrhagic activity of *E. carinatus* venom which was further confirmed by histopathological examinations of mice skin tissue sections.
NNAh, UCB and BD1 also inhibited myonecrosis induced by *E. carinatus* venom and reduced activity of creatine kinase and lactate dehydrogenase in treated animal sera substantiated the anti-myonecrotic effect. NNAh falls into the category of Kunitz-type serine protease inhibitor as determined by peptide mass fingerprinting and shown to be a strong inhibitor of chymotrypsin. UCB and BD1 also inhibited the systemic toxicities induced by *E. carinatus* venom where BD1 was proved to be more potent inhibitor. Systemic alteration of *E. carinatus* venom were assessed by performing routine coagulation assays such as plasma re-calcification time, prothrombin time (PT), activated partial thromboplastin time (APTT) and lethality studies. *E. carinatus* venom pre-incubated with UCB and BD1 showed similar APTT values compared to control, whereas only BD1 group showed values of PT and Plasma re-calcification time similar to control. UCB and BD1 also inhibited the lethal potency of *E. carinatus* venom in both co-injection and challenging studies. Collectively our data signify that NNAh is a Kunitz-type chymotrypsin inhibitor which also inhibited metalloprotease activities of *E. carinatus* venom. In future, complete sequence of NNAh and peptide region(s) responsible for inhibition will assist to deduce the mechanism of action. Further, inhibition offered by UCB and BD1 against proteolytic, hemorrhagic and myonecrotic activity of *E. carinatus* venom will be useful to translate these molecules as therapeutics to treat SVMPs-induced local and systemic manifestations.