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Fractionation and functional characterization of *Bothrops alternatus* (*Rhinocerophis alternatus*) snake plasma: Evaluation of its inhibitory potential on proteolytic enzymes.

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Introduction: Snake venoms are complex mixtures of toxins and enzymes showing several biological activities. They are rich in proteolitic enzymes (serine proteases and metalloproteases) involved in activities such as hemorrhage, coagulation disorders, necrosis and inflammation. The serum, plasma and/or muscles of snakes contain inhibitory compounds against these proteases as a protective mechanism avoiding possible damages caused by their own venoms. Preliminary results of this project showed several fractions obtained from snake plasma with high inhibitory potential on proteolytic enzymes. **Objectives**: This study aimed at the identification of fractions from *B. alternatus* (recently reclassified as *Rhinocerophis alternatus*) snake plasma showing inhibitory potential on the clotting and hemorrhagic activities induced by snake venom serine proteases and metalloproteases, respectively. **Methodology**: Lyophilized *B. alternatus* snake plasma (150 mg) was fractionated on a DEAE-Sepharose column using a FPLC system. For monitoring and selecting the obtained fractions (P1 – P7), clotting and hemorrhagic activities were assayed using snake venom proteases from *Bothrops atrox*. One of the fractions (P3) showing inhibition of the hemorrhagic activity was rechromatographed on a MonoQ 5/50 GL column using HPLC. The obtained fractions (Q1 – Q11) were monitored by SDS-PAGE using 10% polyacrylamide gels, in the presence or absence of β-mercaptoethanol. Clotting activity was determined by the time (in seconds) for the clot formation, using human plasma in the presence of serine proteases from *B. atrox* previously incubated with each one of the obtained fractions. Hemorrhagic activity was evaluated by measuring the hemorrhagic halo observed on mice skin after subcutaneous injection of metalloproteases from *B. atrox* previously incubated with the mentioned fractions. **Results**: Seven fractions (P1-P7) were obtained after fractionation of *B. alternatus* snake plasma on DEAE-Sepharose, showing several bands on SDS-PAGE. All fractions were evaluated regarding their inhibitory potential on the clotting and hemorrhagic activities. Fraction P2 showed a promising inhibition of the clotting activity (40% inhibition, a statistically significant value when compared to the serine protease control). Regarding the hemorrhagic activity, fraction P3 showed high inhibitory potential, as observed by the absence of formation of hemorrhagic halos on mice. When this fraction was rechromatographed on a MonoQ column, 11 fractions (Q1-Q11) were obtained and their hemorrhagic activities were evaluated. Fraction Q4 showed high inhibitory potential (100% inhibition) when compared to the hemorrhagic activity of the metalloprotease control. **Conclusion**: Fractions P2 and Q4 obtained from *B. alternatus* snake plasma showed high inhibitory potential on the clotting and hemorrhagic activities, respectively. Our future studies will aim at the isolation and characterization of the protease inhibitors present in these fractions, generating new information that could lead to new alternatives for serum therapy, which is the only available option for the treatment of snakebites at present.

Keywords: Protease inhibitors, Serine proteases, Metalloproteases, Clotting activity, Hemorrhagic activity.

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