

Purification and Characterization of Inhibitory Peptides of Platelet Aggregation from *Bothrops moojeni* Venom

Sousa, B. B.^{1,4}; Pereira, D. F. C.¹; Matias, M. S.¹; Ribeiro, B. S.¹; Costa, K. C. T.²; Mamede, C. C. N.^{3,4}; Espindola, F. S.¹; Oliveira, F.^{2,4}

¹Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, MG, Brazil; ²Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, MG, Brazil; ³Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, MG, Brazil; ⁴Instituto Nacional de Ciência e Tecnologia em Nano-Biofarmacêutica (N-Biofar), MG, Brazil

INTRODUCTION: *Bothrops* snake venom contains various substances, which interact with components of the human hemostatic system, such as proteins and peptides. These molecules can interfere in platelet aggregation, either inhibit or activate it. **OBJECTIVES:** In this work, we aimed to purify a platelet aggregation inhibitor fraction from *B. moojeni* venom, which was named G3 fraction. **MATERIAL AND METHODS:** The fractionation of *B. moojeni* venom was carried out through two chromatographic steps (ion-exchange on DEAE-Sephacel followed by molecular exclusion on Sephadex G-75). The steps of fractionation were monitored by SDS-PAGE. Aggregation platelet assays were performed in human platelet-rich plasma and measured using an automated four channels aggregometer (AggRAM™ (Helena Laboratories, EUA) at 37°C. Aggregation was triggered with collagen (10 µg/mL), ADP (20 µM) or epinephrine (300 µM) after incubation of platelets with different doses of the G3 fraction (10, 25 and 40µg). One hundred percent (100%) aggregation was expressed as the percentage absorbance relative to platelet-poor plasma aggregation. **RESULTS AND DISCUSSION:** The fractionation of *B. moojeni* venom by ion-exchange chromatography resulted in six main protein fractions, denominated DS1 to DS6. Further chromatography of DS6 fraction on molecular exclusion column resulted in the elution of three main proteins peaks, denominated G1 to G3. The SDS-PAGE analysis showed that the G3 fraction consists of proteins of low molecular weight. This fraction was able to inhibit the aggregation induced by epinephrine in a dose-dependent manner. G3 fraction showed a little or no effect on platelet aggregation induced by collagen or ADP. **CONCLUSIONS:** In this work we purified a fraction of proteins of low molecular weight from *B. moojeni*, which inhibits platelet aggregation induced by epinephrine. It could be of medical interest as a new tool for the development of novel therapeutic agents for the prevention and treatment of thrombotic disorders.

Keyword: Snake venom, *Bothrops moojeni*, platelet aggregation
Supported by: CAPES, CNPq, MCTI, FAPEMIG and UFU