

Molecular cloning and expression of jararin, a new disintegrin from *Bothrops jararaca* venom

David, V.¹; Guerrero, T. N.¹; Magalhães, G.S.⁴; Almeida, F.C. L.¹; Geraldo, R. B.¹; Albano, R. M.³; Wemelinger L. S.²; Zingali, R. B.¹;

¹ Instituto de Bioquímica Médica Leopoldo de Meis, UFRJ, RJ, Brazil;
² Departamento de Análises Clínicas e Toxicológicas, UFRJ, RJ, Brazil;
³ Departamento de Bioquímica, UERJ, RJ, Brazil;
⁴ Instituto Butantan, SP, Brazil;

Introduction and objectives: Snake venoms contain several components, some of which originate drugs and diagnostic tools diseases. Among these components, we find the disintegrins, which are a family of small peptides rich in cysteine. These peptides selectively bind to integrin receptors, which are glycoproteins that play a fundamental role in regulation of physiological and pathological processes, such as hemostasis and thrombosis. Jararin, a disintegrin identified by our group from the cDNAs of the snake Bothrops jararaca gland, which presents an extra cysteine residue at position 14, motivated us to clone and express this new disintegrin with distinct cysteine pattern to analyze if this peptide would carry different selectivity to integrin receptors. The aim of this work was obtain the disintegrin in recombinant form. Material and methods: The sequences were amplified from the cDNA library by PCR. Then, an agarose gel 1.5 % was performed and the DNAs were extracted and digested with restriction enzymes. The products of these reactions were subcloned into pSMT3, then transformed in bacteria Escherichia coli C43. The disintegrin expression was perform in Escherichia coli, incubated at 37°C for 6h. The disintegrin was purified using cobalt resin and the expression was confirmed by SDS-PAGE, Western blotting and mass spectrometry. Results and Discussion: We cloned in pSMT3, a new disintegrin called as jararin. The expression system of the recombinant protein allowed to obtain jararin in its soluble form according to the electrophoresis profile and western blotting, which showed a band with molecular weight around 30 kDa for fusioned jararin. After cleavage and purification, the expression of the disintegrin was confirmed by mass spectrometry with a molecular mass of 7.875,1 Da. Conclusions: Thus, the results in this study allowed to obtain recombinantly a new disintegrin, contributing to obtain biological molecules for their study in different physiological and pathological processes.

Acknowledgements: CAPES, CNPq and FAPERJ. Key words: disintegrin, thrombosis and snake venom.