Enzyme Inhibition Profile and Tissue Localization of a *Rhipicephalus appendiculatus* Cystatin

Konrdörfer, C.R[1]; Sabadin, G.A.[1]; Parizi, L.F.[1,2,7]; Githaka N.W.[2,7]; Nene, V[7]; Seixas, A.[3,6]; Logullo, C.[4,6]; Konnai, S.[2]; Ohashi, K.[2]; da Silva Vaz, I.[1,5,6]

[1]Centro de Biotecnologia, UFRGS, Brazil; [2]Laboratory of Infectious Diseases, Hokkaido University, Japan; [3]Departamento de Farmacociências, UFCSPA, Brazil; [4]Laboratório de Química e Função de Proteínas e Peptídeos, UENF, RJ, Brazil; [5]Faculdade de Veterinária, UFRGS, Brazil; [6]Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular, Brazil; [7]International Livestock Research Institute (ILRI), Nairobi, Kenya.

INTRODUCTION: Cystatins are cysteine protease inhibitors, responsible for blocking the activity of cathepsins, enzymes involved in important physiological processes like digestion, immune response and blood coagulation. Cystatins are present in tick saliva and are released into the attachment site during the parasite feeding process. However, the physiologic role of cystatins from *Rhipicephalus appendiculatus*, a tick that causes economic losses in African livestock, is still unknown. OBJECTIVE: The goal of the present work was to assess the enzyme inhibition profile and tissue localization of a *R. appendiculatus* cystatin (QnRacys2a). MATHERIALS AND METHODS: The QnRacys2a predicted amino acid sequence was analyzed, ORF was cloned and the protein expressed in *Escherichia coli* BL21(DE3)pLysS strain. The recombinant protein was purified by affinity chromatography. Enzymatic assays were conducted to analyze the rQnRacys2a inhibition of bovine cathepsin C and α-chymotrypsin, a serine protease. The immunizations were performed in rabbit and the anti-rQnRacys2a serum was used in western blot assays to determine the rQnRacys2a immunogenicity and distribution of native cystatins in *R. appendiculatus* tissues. RESULTS AND DISCUSSION**:** rQnRacys2a inhibited the activity of cathepsin C with a Ki of 90.9 nM +/- 15.7 nM. The rQnRacys2a did not inhibit the activity of α-chymotrypsin. Western blot showed that rQnRacys2a was immunogenic. Finally, the presence of QnRacys2a was detected in unfed larvae and adults of *R. appendiculatus*, suggesting that this cystatin participates of these development stages. CONCLUSION: QnRacys2a is a cysteine protease inhibitor and is not a serine protease inhibitor, as expected. QnRacys2a presence in different tick stages must be studied to understand its role in *R. appendiculatus* development. The rQnRacys2a inhibitory profile against cathepsin B and L and the effect of antibodies anti-rQnRacys2a on cystatin inhibitory capacity are in evaluation. In addition, the immune response cross-reactivity between the immune response against *R. microplus* cystatins and rQnRacys2a are being evaluated.

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