IN VITRO AND IN VIVO STUDIES OF PMTKI (A KUNITZ-TYPE PROTEASE INHIBITOR FROM Piptadenia moniliformis BENTH) ON INFLAMMATION AND BLOOD CLOTTING: EFFECTS ON STIMULATED NEUTROPHILS, THEIR ELASTASE (HNE) AND ON BLOOD COAGULATION CASCADE

Joelton Igor Oliveira da Cruz ¹; Antônio Moreira Marques Neto¹; Adeliana Silva de Oliveira¹; Iane Costa¹; Luciana Maria Mraujo Rabelo¹; Paula Ivani Medeiros dos Santos¹; Raphael Paschoal Serquiz¹; Elizeu Antunes dos Santos¹.

¹ Departamento de Bioquímica, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, Brazil.

Introduction: Inflammation is a complex biological phenomenon involving large numbers of cells, tissues and macromolecules. One of the protagonists during its acute phase is the polymorphonuclear neutrophil, which lists effector and regulatory functions in the inflammatory process. When activated the cell releases from its granules numerous effectors and immunomodulatory compounds. Among them is the Human Neutrophil Elastase (HNE), serinic proteinase subject of various studies. The catalytic activity regulation of this enzyme occurs, among other ways, by direct and indirect inhibition of endogenous inhibitory proteins. Distribution of proteinase inhibitors in living beings is ubiquitous. In plants they represent a multifunctional arsenal whose performance concerns defense, nutritional reserves and regulation.

Objectives: Evaluate the immunomodulatory ability by direct interaction against neutrophils, inhibition capacity against ENH and Proteinase 3 (another neutrophil proteinase) and rat paw edema prevention. Also, PmTKI toxicity over plasmatic cells and coagulation enzymes inhibition were evaluated. Methods and Results: PmTKI directly inhibited ENH catalytic activity, presenting an IC₅₀ of 5 µg.mL⁻¹. Also, PmTKI prevented ENH release from neutrophil when stimulated with PAF and fMLP (endo- and exogenous stimulants, respectively) by 56% and 42%. It also presented low reduction on weight measures on edema formation in carrageenan-induced rats. PmTKI slightly inhibited Proteinase 3 activity and had no interference over Thrombin and activated Factor X. The Cephalin-Kaolin Time was prolonged (dose-dependent), while no change in Quick Time (aPTT and PT tests, respectively) was showed. PmTKI showed no cytotoxicity to human red blood cells (hemolysis assay) neither to white blood cells (flow citometry).

Conclusion: These results indicate PmTKI as a potential candidate for drug development in the therapy of inflammatory disorders, especially those with relevant neutrophil participation.

Keywords: PmTKI; Inhibitor; Coagulation.

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