Fluorescence Spectroscopy Interaction Study Between hRSV NS1 Protein and Flavonoids

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INTRODUCTION: Human Respiratory Syncytial Virus (hRSV) is the major causative of acute respiratory infections in children, like bronchiolitis and pneumonia. This Paramyxovirus has a single strand RNA with 10 genes that codify 11 proteins. An important factor that contributes for the success in hRSV replication is the its immune system evasion, process provided by Non-Structural Protein 1 (NS1). NS1 is a 139 amino acids and 15kDa protein. This protein can act inhibiting or neutralizing several steps of IFN path, as well as, act silencing the ribonucleoproteic complex of hRSV. The knowing of NS1 protein interaction with ligands is important to propose a mechanism to inhibit infection and flavonoids are described to being viral replication suppressors. The aim of this study includes expression and purification of NS1 protein to perform fluorescence spectroscopy interaction study between NS1 and the flavonoids Quercetin, Miricetin, Kaempferol, and Kaempferol-3β-D-glucoyranoside.

MATERIAL AND METHODS: The plasmid construction pJexpress401-NS1 was transformed into BL-21(DE3) bacteria and expressed using 0.5mM IPTG. Purification was developed in affinity chromatography (nickel resin), with an imidazole gradient (40-500mM). Following, concentration of protein and flavonoids were determinated by UV-Vis spectroscopy and the interaction tests were performed trough fluorescence spectroscopy titration. The values obtained were used to calculate the binding (Kb) constant and number of ligands per site (n).

RESULTS AND DISCUSSION: Results shown the expression of protein with 16,36kDa and better condition of elution purification around imidazole 500mM. The Kb obtained were in order of 10⁻⁵M⁻¹, what indicate a strong interaction and the preference ordering were given for Miricetin> Quercetin> Kaempferol-3βD-glucoyranoside> Kaempferol. The results showed that NS1 has one binding site for each flavonoid. CONCLUSION: The data reveals there are interaction between NS1 and flavonoids, and thus, flavonoids can act like a inhibition for NS1, blocking its function of immune system subversion.

Key words: hRSV, NS1 Protein, Flavonoids, Fluorescence Spectroscopy.
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