BIO_03 - Development of a therapeutic strategy for COVID-19 based on angiotensin-converting enzyme 2 (ACE-2) recombinant proteins

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Introduction: SARS-CoV-2 virus causes COVID-19. Although several vaccines have been approved for emergency use, there is a threat of new variants of concern followed by immune escape, which leads to search for new strategies against this virus. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE-2) as a receptor binding to infect host cells. Therefore, ACE-2 becomes a good target to neutralize the viral particle *in vivo*.

Objective: Structural characterization and measure the ability of recombinant ACE-2 Fc and ACE-2 Strep His proteins neutralize SARS-CoV-2 *in vitro*.

Methodology: Size Exclusion Chromatography (SEC) evaluated recombinant ACE-2 proteins. Structural characterization was performed by Intrinsic Tryptophan Fluorescence (ITF) and Circular Dichroism (CD) analysis, including kinetics of thermal denaturation at range 25-85°C and Dichroweb calculations. In neutralizing step, SARS-CoV-2 was added to growing dilutions of ACE-2 proteins and incubated with Vero cell monolayers. Supernatant was replaced by semi-solid median and incubated for 3 days. Plaques were counted or supernatant/cells were collected to qPCR analyses.

Results: SEC analysis of ACE-2 Fc showed a profile with three main peaks, suggesting aggregation. Surprisingly, ACE-2 Strep His profile showed a major peak at 278.7kDa (86.8% homogeneity), suggesting a tetramer form. ITF and CD thermograms showed conformational stability until 40°C for ACE-2 Fc and 45°C for ACE-2 Strep His, whose CD spectrum revealed 41.1% and 42.5% of unordered structure. Both ACE-2 proteins were able to induce 100% of SARS-CoV-2 neutralization *in vitro* (up to 50µg/mL) and a high percentage of neutralization (~ 80%) was found in dilutions up to 1.56µg/mL, with low RNA copy nº/mL in dilutions up to 12.5µg/mL.

Conclusion: While structural analysis demonstrated unordered structure, both proteins were able to neutralize completely the SARS-CoV-2 infection *in vitro*. Fc portion of IgG1 fused to ACE-2 did not interfere with *in vitro* neutralization efficiency and could prolong the half-life of ACE-2 *in vivo*. Next steps involve *in vivo* evaluations in K-18 mice and biomolecular interaction by microscale thermophoresis.

Keywords: SARS-CoV-2; Virus neutralization; Structural characterization