BIO_17 - Characterization of the anti-SARS-CoV-2 activity of hypericin in an *in vitro* infection model

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Introduction: The COVID-19 pandemic has had a major impact on public health around the world, with alarming consequences for human mortality and morbidity. Currently, some treatment options for COVID-19 are available, however, they may have some limitations. In this sense, the search for new, more potent antiviral compounds or those with immunomodulatory and anti-inflammatory properties is essential to expand therapeutic options. Furthermore, with the emergence of new variants, resistance mutations may emerge that lead to the ineffectiveness of drugs that have been used. Studies show that the compound hypericin has anti-SARS-CoV-2 activity, requiring further *in vitro* analyzes to understand its antiviral mechanism of action.

Objectives: Therefore, we propose to characterize the activity of hypericin with respect to its ability to inhibit the replication of the SARS-CoV-2 virus in vitro and identify its mechanism of antiviral action.

Methodology: To do this, we used Vero E6 cells infected with the original SARS-CoV-2 isolate and treated with increasing concentrations of hypericin for 48 hours. To evaluate the ability to reduce viral replication, the cell supernatant was titrated by plaque assay, 48 hours post-infection.

Results: Thus, we observed a significant reduction in the viral titer in a dose-dependent manner, with hypericin IC50 of 493.4 pg/ml. To evaluate the mechanism of action, we performed a drug addition time assay, at a concentration of 10 ng/ml, under the following conditions: total treatment (1), pre-treatment of cells (2), pre- treatment of viruses at 4 °C, 20 °C and 37 °C (3), treatment during infection (4) and post-infection treatment (5). Condition 1, used as a comparison parameter, showed a total reduction in viral titers. No change in viral replication was observed in condition 2, indicating that hypericin would not block cellular components that have viral interaction. For conditions 3 and 4, a significant reduction in viral titer was observed, indicating the possible virucidal activity of the drug regardless of temperature. Despite this, under these conditions, the titer reduction was smaller than in condition 1. Condition 5 also showed the antiviral activity of hypericin at its maximum effectiveness, as it completely inhibited viral replication.

Conclusion: Thus, we conclude that hypericin has important antiviral and virucidal activities. Our studies open a new perspective for the inhibition of SARS-CoV-2 infection, with the characterization of a new antiviral compound and its mechanism of action.

Keywords: Hypericin; SARS-CoV-2; Antiviral