Title: INHIBITION OF SARS-COV-2 REPLICATION BY HYPERICIN IN AN IN VITRO INFECTION MODEL

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Introduction: COVID-19 pandemic had a huge impact on global public health. Although some treatments against this disease are available, they have limitations. Additionally, the continuous emergence of viral variants may lead to mutations that result in antiviral resistance, reducing the effectiveness of current medications. Therefore, the investigation of new antivirals inessential to expand therapeutic options. Objectives: We propose the investigation of the activity of hypericin (HY) in inhibiting the replication of SARS-CoV-2 and to clarify its mechanism of action in an *in vitro* infection model. Methodologies: We used Vero E6 cells infected with distinct SARS-CoV-2 variants and treated these cells with different concentrations of hypericin for 48 hours. To evaluate the drug mechanism of action, infected Vero E6 cells were treated in different time points, as follows: total treatment (1), pretreatment of the cells (2), pretreatment of the viruses at 4°C, 20°C and 37°C (3), treatment during infection (4) and treatment post-infection (5). In addition, we analyzed the efficacy of the combination of HY with remdesivir (RDV) and/or nirmatrelvir (NMV). Viral replication was assessed through viral titration. Results: We observed a significant reduction in viral titer in a dose-dependent manner for the different variants of SARS-CoV-2, with HY IC_{50} = 1 nM. To understand HY mechanism of action, condition 1 showed a total reduction in viral titers. In condition 2, we observed no changes in viral replication, suggesting that HY does not block cellular components essential for viral interaction. Conditions 3 and 4 resulted in a significant reduction in viral title, indicating a possible virucidal activity of the drug, regardless of temperature, although the reduction was less relevant than in condition 1. Condition 5 also demonstrated the maximum antiviral efficacy of HY. For drug combinations, we observed that double combinations (HY and RDV or HY and NMV) indicated a synergistic effect of HY with both drugs, resulting in a significant reduction in viral titers. However, the triple combination (HY and RDV and NMV) showed an antagonistic effect between the drugs. Conclusions: HY has important antiviral and virucidal activities against a broad of SARS-CoV-2 variants, offering a new perspective for COVID-19 treatment in an *in vitro* model, and characterizing a new antiviral compound and its combinations with previously approved drugs for this disease treatment.

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