Photodynamic Inactivation of Bovine Coronavirus with the Photosensitizer Toluidine Blue O

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Coronaviruses (CoVs) belong to the group of enveloped positive-sense single-strand RNA viruses and are causative agents of respiratory, gastro-intestinal, and central nervous systems diseases in many host species, i.e., birds, mammals, and humans. Beta-CoVs revealed a great potential to cross the barrier between species by causing three epidemics/pandemics among humans in the 21st century. Considering the urgent need for powerful antiviral agents for decontamination, prevention, and treatment of BCoV infections, we turned our attention to the possibility of photodynamic inactivation with photosensitizers in combination with light irradiation. In the present study, we evaluated, for the first time, the antiviral activity of toluidine blue O (TBO) against Beta-coronavirus 1 (BCoV) in comparison to methylene blue (MB). First, we determined the in vitro cytotoxicity of MB and TBO on the Madin–Darby bovine kidney (MDBK) cell line with ISO10993-5/Annex C. Thereafter, BCoV was propagated in MDBK cells, and the virus titer was measured with digital droplet PCR, $TCID_{50}$ assay and plaque assay. The antiviral activity of non-toxic concentrations of TBO was estimated using the direct inactivation approach. All effects were calculated in MAPLE 15® mathematical software by developing programs for non-linear modeling and response surface analysis. The median inhibitory concentration (IC_{50}) of TBO after 72 h of incubation in MDBK cells was 0.85 µM. The antiviral activity of TBO after the direct inactivation of BCoV (MOI = 1) was significantly stronger than that of MB. The median effective concentration (EC_{50}) of TBO was 0.005 µM. The cytopathic effect decreased in a concentration-dependent manner, from 0.0025 to 0.01 µM, and disappeared fully at concentrations between 0.02 and 0.3 µM of TBO. The number of virus particles also decreased, depending on the concentration applied, as proven by ddPCR analysis. In conclusion, TBO exhibits significant potential for direct inactivation of BCoV in vitro, with a very high selectivity index, and should be subjected to further investigation, aiming at its application in veterinary and/or human medicinal praxis.