Supplementary Information

Reviving chloroquine for anti- SARS-CoV-2 treatment with Cucurbit[7]uril-based supramolecular formulation

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Materials and methods

Materials. CB[7] was synthesized and purified according to a reported method.[1] Chloroquine diphosphate (CQ) was purchased from TCI (Shanghai) Development Co., Ltd. Cell counting kit-8 (CCK-8) was purchased from Beyotime.
Nuclear Magnetic Resonance (NMR) Titration. CQ (1.0 mM) solution in D_2O were used to dissolve different equivalent (eqv.) of CB[7] (0.5 eqv., 1.2 eqv. and 2.2 eqv.). ^{1}H NMR spectra was acquired by using Bruker 600 MHz NMR spectrometer.
Job plot. Aqueous solutions of CB[7] (0.025 mM) and CQ (0.025 mM) were separately prepared. Testing samples were prepared by mixing different volumes of CB[7] and CQ solution, with ratio of CQ/(CQ+CB[7]) ranging from 0 to 1.0 (intervals 0.1). All samples were scanned by A HACH DR6000 UV-Vis spectrometer. After collecting the absorption data of all samples, the absorption at 330 nm wavelength was selected for the analysis of ΔA.
Electrospray Ionization Mass Spectrometry (ESI-MS). ESI-MS was used to study the binding ratio of the host-guest complexes. Samples were obtained from the dilution of the mixture of CQ (1.0 mM) and CB[7] (2.0 mM) solutions. The sample was sonicated and filtered before being injected into Thermo LTQ Orbitrap XL mass spectrometer.
Isothermal Titration Calorimetry (ITC). ITC was used to study the binding information (binding ratio and binding constant) of the host-guest complexes. In the experiment, CQ (0.1 mM) and CB[7] (2.0 mM) were separately prepared by ultrapure water and sonicated before the experiment. The titration was conducted by titrating 19 drops of CB[7] solution to CQ solution. ITC analysis was performed using MicroCal PEAQ ITC Analysis Instrument. The heat generated from CB[7] dilution was subtracted as control. The 'one set of sites' isotherm model was used to analyse the result.
Cell viability assay. Vero E6 and L-02 cells were separately seeded into 96 well cell culture plates at 1×10^4 cells per well for 24 h till attachment. Then, cells were treated with different concentration of CQ and CB[7] in non-serum medium for 24 h and 48 h. Next, cells were washed and were incubated for 3 h at 37 ^{o}C in CCK-8 solution. Absorbance was tested by using Spectra Max M5 Microplate Reader at 450 nm. Cell viability data were presented by percentage relative to the control group.
Virus. The nCov-2019BetaCoV/Wuhan/WIV04/2019 was propagated in Vero E6 cell line. The viral titer was determined by 50% tissue culture infective dose (TCID50) using immunofluorescence assay. All the infection experiments were performed in a biosafety level-3 laboratory. MHV-A59 (GenBank accession: AY700211.1) was cultured in Vero E6 cell line. The viral titer was determined by viral plaque assay. All the infection experiments were performed in a biosafety level-3 laboratory.
Antiviral activities. Vero E6 cells were seeded into 24 well cell culture plates at 5×10^4 cells per well for 24 h till attachment. Then, cells were infected with SARS-CoV-2 or MHV at a multiplicity of infection (MOI) of 0.05 for 1h with different concentrations of drugs as indicated for 1h. Next, the virus-drug supernatant was removed and the cells were washed with PBS twice, and cell culture medium with drugs as indicated were added. At 24 h post infection, the supernatant was collected for further experiment.
N2A cells were seeded into 24 well cell culture plates at 5×10^4 cells per well for 24 h till attachment. Then, cells were infected with MHV-A59 at an MOI of 0.01 for 1h with different concentrations of drugs as indicated for 1h. Next, the virus-drug supernatant was removed and cells were washed with PBS twice, and cell culture medium with drugs as indicated were added. At 24 h post infection, the supernatant was collected for further experiment.
Quantitative real-time PCR (qRT-PCR). The viral RNA extraction and qRT-PCR were performed as previously described.[2] Cell culture supernatant was harvested for viral RNA extraction by using the MiniBEST Viral RNA/DNA Extraction Kit. RNA was eluted in 30 μL RNase-free water. Reverse transcription was performed with a PrimeScript RT Reagent Kit with gDNA Eraser and qRT-PCR was performed on StepOne Plus Real-time PCR system with TB Green Premix Ex Taq II. Briefly, 3 μL total RNA was first digested with gDNA eraser to remove contaminated DNA and then the first-strand cDNA was synthesized in 20 μL reaction while 2 μL cDNA was used as template for quantitativePCR. Receptor binding domain (RBD) of spike gene was amplified by PCR from the cDNA template with primers: RBD-F: 5'-GCTCCATGGCCTAATATTACAAACTTGTGCC3'; RBD-R: 5'-TGCTCTAGACTCAAGTGTCTGTGGATCAC-3'; and cloned into pMT/BiP/V5-His vector (Invitrogen). It was used as the plasmid standard after its identity was confirmed by sequencing. A standard curve was generated by determination of copy numbers from serially dilutions (103-109 copies) of the plasmid. The primers used for quantitative PCR were RBD-qF1: 5'-CAATGGTTTAACAGGCACAGG-3' and RBD-qR1:5'-CTCAAGTGTCTGTGGATCACG-3. PCR amplification was performed as follows: 95 oC for 5 min followed by 40 cycles consisting of 95 oC for 15 s, 54 oC for 15 s, 72 oC for 30 s. The dose-response curves were plotted form viral RNA copies versus the drug concentrations by using Graphpad Prism 6 software.[3]

Immunofluorescence microscopy. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Then the cells were blocked with 5% bovine serum albumin (BSA) at room temperature for 2 h. The cells were further incubated with the primary antibody (a polyclonal antibody against viral nucleocapsid protein [abbreviated as anti-NP] of a bat SARS-related CoV,1:1000 dilution) 1 for 2 h, followed by incubation with the secondary antibody (Alexa 488-labeled goat anti-rabbit [1:500; Abcam]). The nuclei were stained with Hoechst33258 dye (Beyotime, China). The images were taken by fluorescence microscopy.

Viral plaque assay. Vero E6 cells were cultured to determinate viral titer. Briefly, serial 10-fold dilutions of viruses were added into monolayer cells. After adsorption at 37 ºC, the virus inoculum was removed and cells were washed with PBS twice, then DMEM containing 5 % FBS and 1.0 % methylcellulose was supplemented. Plates were incubated for 2 days until obvious plaques could be observed. Cells were stained with 1% crystal violet for 4h at room temperature. Plaques were counted and viral titers were expressed as PFU/ml. The dose-response curves were plotted form viral RNA copies versus the drug concentrations by using Graphpad Prism 6 software.

References

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