SUPPLEMENTARY MATERIALS

A Phase II, randomised, double-blind, placebo-controlled study to evaluate the efficacy of HEX17, a novel broad-spectrum antiviral drug, in a controlled human infection model of influenza challenge

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RT-qPCR sample preparation

Samples were prepared for RT-qPCR analysis using the QIAsymphony DSP Virus/Pathogen Midi Kit (QIAGEN, Hilden, Germany) in the QIAsymphony instrument (QIAGEN) per the manufacturer's instructions, with automated sample preparation and assay setup. Positive extraction control (spiked matrix containing Influenza A/Perth) and a negative extraction control with matrix only were included in each extraction. Mouse globin was used as an internal control. For the nucleic acid extraction, 1 mL per sample was used and eluted into 60 µL (30 µL was used for the assay; 10 µL for each of three replicates). All RT-qPCR assays used the QuantiFast Multiplex RT-PCR +R Kit (2000) (QIAGEN), per the manufacturer's instructions, and were performed using the ViiA™7 thermocycler (Applied Biosystems, Massachusetts, USA). All samples were measured in triplicate. Data were analysed using ViiAnalyse (in-house software for transposing and analysis of data).

Viral culture

For viral culture, Madin-Darby Canine Kidney cells grown in culture media (1X Eagle's Minimum Essential Media, 5% heat-inactivated foetal bovine serum, 100 units/0.1 mg penicillin/streptomycin, 10 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid [HEPES] buffer, 1X non-essential amino acids, and 2 mM L-glutamine) were seeded in a 96-well flat bottomed plate at a density of 5×10^4 cells/mL per well in 100 µL volume. Cells were incubated for approximately 24 hours (37ºC, 5% CO_2) until they were 50% to 90% confluent. Media was removed and cells were washed twice with 100 µL pre-warmed (37ºC) influenza infection media (1X Dulbecco's Modified Eagle Medium, 100 units/0.1 mg

penicillin/streptomycin, 10mM HEPES buffer, 1X non-essential amino acids, and 1 μg/mL trypsin L-1-tosylamide-2-phenylethyl chloromethyl ketone [Thermo Scientific, Massachusetts, USA]). After washing, 100 μl pre-warmed influenza infection media was added to each well, and plates were incubated (37ºC, 5% CO_2) for up to 8 hours. Test samples were thawed immediately before use and kept on wet ice during the assay. An 11 μL volume of test sample was added to the first row of each plate; samples were usually assessed in quadruplicate. A Log_{10} serial dilution series was performed as follows: 11 μL from the first infection row was added to the corresponding wells in the second row; after mixing, 11 μL from the second row was added to the corresponding row in the third row, and so on, for a total of seven rows. Plates were incubated for approximately 72 hours (37ºC, 5% CO_2). The same plating and serial dilution were performed for the influenza virus control. No test sample was added to a row of cells as a negative control. Virus concentrations were measured by haemagglutination assay. First, 50 μL of supernatant were removed per well and transferred to another 96 well V-bottomed microtitre plate. A 50 μL aliquot of 0.5% Turkey red blood cells in phosphate buffered saline was added to each well, and cells were incubated for approximately 30 minutes. Turkey red blood cell agglutination was assessed by eye, and virus infectious titre was calculated using the Karber calculation [1].

REFERENCES

1. Karber G. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie 1931;162(4):480–3.