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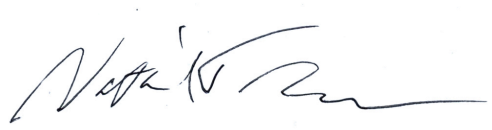
To whom it may concern,

Please find attached the results of our experiments to measure the impact of METAS solution on the replication of Murine Hepatitis Virus (MHV), a model system for SARS-CoV-2 replication. Our data indicate that METAS solutions > 0.3125% inhibit MHV replication at concentrations that do not affect the viability of uninfected cells. Detailed experimental protocols and results are included in the attached document.

Sincerely,



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Experimental Design and Background

The METAS solution was tested for its ability to prevent cytopathic effect (CPE) caused by Murine Hepatitis Virus (MHV), a close relative of pathogenic human coronaviruses including SARS-CoV2, the causative agent of COVID-19 disease.

MHV infection causes a characteristic change in the morphology of infected cells, leading to the formation of large multi-nucleated cells, or syncytia, which can be easily recognized under a microscope after 24 hours of infection. The impact of METAS solution on MHV infection was determined by measuring the ability of different METAS concentrations to prevent syncytia formation caused by MHV in DBT cells. DBT cells were originally derived from a murine astrocytoma, and are commonly used in the study of MHV replication. The gross effect of METAS solution on cell viability was also measured in uninfected cells.

Methods

DBT cells were cultured in growth media (DMEM containing 10% fetal bovine serum (FBS)), and then plated in 96 well plates such that the cells were 80% confluent at the time of infection. The media was removed from the wells, and replaced with 50µl of DMEM+10%FBS containing various amounts of MHV. METAS solution was diluted in DMEM+10% FBS to achieve final concentrations of 10%, 2.5%, 0.625%, 0.156%, 0.039%, 0.009%, 0.0024%, and 0.00061%. 50µl of each dilute METAS solution was added to each well of a row of a 96 well plate, such that 12 wells of the plate were treated with each drug concentration in the presence of virus. As the METAS solution was mixed with an equal volume media containing virus solution, the final concentration of METAS tested were 5%, 1.25%, 0.3125%, 0.078%, 0.0195%, 0.0045%, 0.0012%, and 0.0003%. Equivalent dilutions of water were tested for their effect on MHV induced CPE as a negative control. In parallel, uninfected cells were treated with the same final concentrations of METAS solution to measure the effect of the compound on cell viability. Each experiment also included at least one 96 well plate of DBT cells that were left uninfected and were not treated with any compound (water or METAS) to ensure that any observed effects on cell morphology or viability were not the result of cell culture contamination.

The effect of the compound on MHV replication was measured by scoring each well for the presence or absence of syncytia by microscopy at 24 hours after infection. The gross effect of the compound on cell viability was examined by microscopy as well.

The antiviral effect of test compounds can vary based on the amount of virus present in the assay. Therefore the experiment was repeated with three different amounts of virus. Infections were performed at a multiplicity of infection (MOI) of 1 infectious unit per cell, 0.1 infectious unit per cell, or 0.01 infectious units per cell. In all cases the assay was read at 24 hours after infection.

Results

Infected cells treated with negative control compound (water) displayed pronounced CPE at all dilutions tested, as expected. Complete protection from CPE was observed in all infected wells treated with 5%, 1.25% or 0.3125% METAS solution diluted in media. No protection from CPE was observed in cells treated with 0.078%, 0.0195%, 0.0045%, 0.0012%, or 0.0003% METAS solution. The same results were obtained at MOIs of 1.0, 0.1, and 0.01. The experiments were repeated three times with identical results in each.

No gross effect on cell viability was observed at any concentration of METAS solution in uninfected DBT cells. METAS solutions of 5%, 1.25%, 0.3125%, 0.078%, and 0.0195% caused the media to immediately change color from pink to a bright yellow, indicating a significant decrease in the pH of the media, though the pH was not directly measured. However cells remained fully viable at all concentrations of METAS when examined at 5 minutes, 24 hours, or 48 hours after the addition of METAS. However decreased cell growth was observed at 48 hours after the addition of at METAS concentrations of 5%, 1.25%, or 0.3125%. While control cells had grown to confluence in 48 hours, cells treated with 5%, 1.25%, or 0.3125% METAS solution remained subconfluent, suggesting these concentrations of METAS impaired cell replication or growth, but did not affect cell viability.

Conclusions

The data show that concentrations of METAS greater than or equal to 0.3125% inhibited CPE caused by the coronavirus MHV without gross affects on cell viability. This suggests that METAS concentrations greater than or equal to 0.3125% inhibit MHV replication.

Caution should be used when considering the potential mechanism of action of METAS in MHV inhibition. The assay used cannot discriminate between effects of METAS on virus integrity, virus attachment and entry, or virus replication in the cell. It is also possible that METAS does not block virus replication, but rather prevents the ability of MHV to induce syncytia formation. However given the complete lack of CPE and the continued viability of infected cells at METAS concentrations greater than or equal to 0.3125%, these results suggest that METAS inhibits MHV replication at a step prior to virus assembly and egress.

Signature:  Date: 07/27/20

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