In Vitro Activity of Cysteamine Against SARS-CoV-2 Variants Alpha, Beta,Gamma and Delta

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Abstract

Global COVID-19 pandemic is caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Continuous emergence of new variants and their rapid spread are jeopardizing vaccine countermeasures to a significant extent. While currently available vaccines are effective at preventing illness associated with SARS-CoV-2 infection, these have been shown to be less effective at preventing breakthrough infection and transmission from a vaccinated individual to others. Here we demonstrate broad antiviral activity of cysteamine HCl *in vitro* against variants of SARS-CoV-2 assayed in a highly permissible Vero cell line. Cysteamine HCl inhibited infection of alpha, beta, gamma and delta variants effectively and the inhibitory activity was shown to manifest during the early stages of viral infection. Cysteamine is a very well-tolerated US FDA-approved drug used chronically as a topical ophthalmic solution to treat ocular cystinosis in patients who receive it hourly or QID lifelong at concentrations 3 to 4 times higher than that required to inhibit SARS CoV-2 in tissue culture. Application of cysteamine as a topical nasal treatment can potentially : 1) mitigate existing infection 2) prevent infection in exposed individuals, and 3) limit the contagion in vulnerable populations..

Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) first emerged in humans during late 2019 in Wuhan, China, and transmitted globally leading to the Coronavirus Disease 19 (COVID-19)

pandemic. COVID-19 has already in resulted in 4,662,980 deaths globally from 42,160,073 infections and the numbers are still growing due to the emergence of new infectious variants. SARS-CoV-2 is a highly contagious virus transmitted via respiratory droplets between humans, and the infection typically presents a wide range of clinical outcomes from the asymptomatic state to respiratory failure leading to multiorgan failure and death in severe cases. Because of the severity of infection noted with SARS-CoV-2 , significant effort been made to develop both vaccine and therapeutic strategies to combat infection. While several vaccines have been developed and administered globally, the effectiveness of such vaccines against the emergent variants have come into question. Moreover, hesitancy to be vaccinated has also complicated global effort in successfully controlling the pandemic. Clearly highly effective therapeutic strategies are needed which, along with vaccines, is may control the pandemic.

While few antivirals have been shown to inhibit SARS-CoV-2 in vitro, successful application of these antivirals in clinical setting has not shown promise. Hence development of highly effective antivirals is clearly needed. Cysteamine, 2-aminoethanethiol, is a simple aliphatic compound which was first used in man as an antidote to acetaminophen poisoning (1). It was subsequently developed as a treatment for cystinosis after it was found to deplete cultured cystinosis fibroblasts of stored lysosomal cystine, which is the hallmark of cystinosis. Cystinosis is inherited as an autosomal recessive inborn error of lysosomal cystine transport, and characterized chiefly by failure to thrive, progressive renal failure and ESRD by age 10 years (2). In 1994 FDA approval was granted for cysteamine treatment of cystinosis and it also received FDA designation as one of the first Orphan Products (3). Currently, Cysteamine is FDA approved and administered to humans in different forms for various treatments. Cysteamine is given orally in the systemic treatment of cystinosis. The usual oral dose in children is 50-60 mg/kg/d, and 1.3-1.6g/m^2/d in adults. These doses yielded peak blood cysteamine concentrations of ~ 50-70 µM 60 min after a po dose (4,5). Second the corneal keratopathy of cystinosis is treated with cysteamine eyedrops (6). Additionally, cysteamine has been administered

intravenously to two cystinosis patients for 1-10 months (7,8), and it is marketed as a 5% cream to treat melasma (9).

Approximately 800 patients in the United States have nephropathic cystinosis and are on cysteamine therapy, which has become the standard of care since FDA's 1994 approval. As a genetic disease, cysteamine is required life-long to treat the renal and extra-renal manifestations of cystinosis which include, in addition to progressive renal failure and corneal keratopathy, distal myopathy, neurocognitive disorders, pulmonopathy, endocrinopathy, diabetes, and metabolic bone disease (10). The general incidence is 1/100,000 live births, although some populations have a higher incidence (11). Prior to approval of cysteamine for treatment of cystinosis, the average native kidney survival was <10 years. Currently, with cysteamine, native renal survival can be expected to age 20 years.

Cysteamine has been studied as antiviral agent. Earlier studies have shown that cysteamine was able to inhibit infectivity of HIV-1 in vitro primarily by inhibiting the binding of gp120 with CD4 lympohocytes (12-15). Moreover, recently it has been shown that cysteamine is also capable of inhibiting infectivity of SARS-CoV-2 presumably by inhibiting the binding of S1 protein to the ACE-2 receptor (16). In this report we examined the inhibitory activity of cysteamine against several variants of SARS-CoV-2 and showed that the compound can inhibit the infectivity of the virus when present during the initiation of infection.

Methods

Cells and Virus

Vero-TMPRSS2 cells used in the infection assay were provided by the Vaccine Research Center (NIH). The use of this cell line in SARS-CoV-2 infection assay is described elsewhere (17). Cells were maintained in Dulbecco's modified Eagle´s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, 1% penicillin/streptomycin and puromycin (10 µg/ml). Wild type and the variants stocks of SARS-CoV-2 were expanded from the seed stocks by infecting Calu-3 cells by

in EMEM medium containing 2% FBS, L-glutamine and penicillin/streptomycin. Virus was isolated by harvesting the cell free culture supernatant three to four days after infection depending upon the amount of the nucleocapsid (NP) protein present in the supernatant as measured by the antigen capture kit (My BioSource). Expanded stocks were shown to be free from the adventitious agent. Identity of each stock was confirmed by deep sequencing. Viruses used in this study include: wild type SARS-CoV-2 (P4) isolate USA-WA1/2020 from BEI resources NR-52281; alpha variant CoVID-19 (2019-nCoV/USA/CA_CDC_5574/2020) from BEI Resources NR-54011; beta variant CoVID-19 (2019-nCoV/South Africa/KRISP-K005325/2020) from BEI Resources NR-54974; gamma variant CoVID-19 (hCoV-19/Japan/TY7-501/2021) TY7-503 p1 (Dr. Takaji Wakita, National Institute of Infectious Diseases, Japan); delta variant hCOV-19/USA/PHC658/2021 (B.1.617.2) from BEI Resources NR-55612. Infectivity of expanded stocks was determined by plaque forming assay in Vero-TMPRSS2 cells. Infectivity titer (pfu/ml) of each stock used in this study was $3.7x10^7$ pfu/ml for wild type, $1.3x10^6$ pfu/ml for alpha; $4.9x10^7$ pfu/ml for beta, $1.8\ x10^7$ pfu/ml for gamma and $2.2\ x10^7$ pfu/ml for delta variant.

Cytotoxicity assay

For evaluating cytotoxicity of cysteamine HCl (ACIC Pharmaceuticals Inc. Brantford, Canada), Vero-TMPRSS2 cells (25000 cells/well) were plated overnight in a 96 well plate in Dulbecco's modified Eagle´s medium (DMEM) supplemented with 10% FBS, L-glutamine, 1% penicillin/streptomycin and puromycin (10 µg/ml). In order to duplicate the method used in virus inhibition assay, varying concentrations of cysteamine HCl were preincubated with Dulbecco's modified Eagle´s medium (DMEM) supplemented with 2% FBS, L-glutamine, 1% penicillin/streptomycin and puromycin (10 µg/ml) (complete DMEM medium) for 2 hours at 37°C in 5% CO2 in 100 µl medium and then transferred to the wells containing Vero-TMPRSS2 cells. After 1 hour of incubation, medium from each well was removed and 100 µl of complete DMEM medium containing either no cysteamine HCl or 20% of the original concentration of cysteamine HCl was added to mimic the condition of the infection assay method and the plates were cultured for 72 hours at 37°C in 5% CO2. Cytotoxicity

was measured by adding 100 µl of Cell titer glow (Promega) reagent and incubated for 15 minutes at room temperature. Luminescence endpoint was read in a plate reader (Biotek Cytation 5). Percent cytotoxicity was calculated based on the luminescence reading of cysteamine HCl-treated wells compared to the medium only treated control wells.

Thiol Quantitation

Measurement of free sulfhydryl group concentration in the infection assay was performed in Vero-TMPRSS2 cells (25000 cells/well) plated overnight in a 96 well plate in Dulbecco's Modified Eagle´s Medium (DMEM) supplemented with 10% FBS, glutamine, 1% penicillin/streptomycin and puromycin (10 µg/ml). The free sulfhydryl concentration at different stages of the infection was determined by Ellman's reagent (5,5'-dithio-bis-2-nitrobenzoic acid, DTNB, Sigma) by rmeasuring the optical density at 412 nm in a plate reader (Biotek Cytation 5) as described elsewhere (https://www.bmglabtech.com/ellmans-assay-for-in-solution-quantification-of-sulfhydryl-groups/). Concentration of free cysteamine HCl was calculated based on the standard curve generated with the known concentrations of cysteamine HCl.

Virus inhibition assay

Virus infection assay by plaque formation was used for evaluating antiviral activity of cysteamine HCl. This assay was performed in a biosafety level 3 facility under compliance with BIOQUAL's health and safety procedures established for work in BSL-3 laboratory. Vero-TMPRSS2 cells (175,000 cells per well) were added into 24 well plates in DMEM medium containing 10% FBS, L-glutamine, puromycin (10 µg/ml) and penicillin/streptomycin and the plates were cultured overnight at 37°C in 5% CO2. For assays measuring dose dependent inhibition of infection, SARS-CoV-2 was preincubated with different concentrations of cysteamine HCl at 37°C for 2 hours in a total volume of 600 µl of complete DMEM medium. Cysteamine HCl/virus mixture was then transferred to each well of a 24 well plate of Vero-TMPRSS2 cells in a total volume of 250 µl and incubated for 1 hour at 37°C in 5% CO2. Each well was then overlaid with 1 ml of culture medium containing 0.5% methylcellulose and incubated for 3 days at 37°C in 5% CO2. The plates were subsequently fixed with methanol at -20°C for 30 minutes

and stained with 0.2% crystal violet for 30 minutes at room temperature. Plaques in each well were manually score. Inhibitory potency measured as absolute IC50 was defined as the concentration of cysteamine HCl that resulted in 50% reduction in the number of plaques compared to the untreated controls. The Absolute IC50 values were calculated using GraphPad Prism 9 program choosing nonlinear regression in a Dose-Response curve. For evaluating the kinetic of virus inactivation both 5 mM and 10 mM concentrations of cysteamine HCl were pre-incubated with SARS-CoV-2 for 0, 15, 30, 60 and 90 minutes and the mixture was then transferred to Vero-TMPRSS2 cells (175,000 cells per well) previously cultured overnight in a 24 well plate in DMEM medium containing 10% FBS, glutamine, puromycin (10 µg/ml) and penicillin/streptomycin. The remaining steps of the infection assay were as described above.

Results

Toxicity of cysteamine HCl in culture

Cytotoxicity of cysteamine HCl in Vero-TMPRSS2 cells was determined to select the concentrations to be used in the dose response inhibition assay. Selected concentrations of cysteamine HCl were preincubated with medium for 2 hours followed by one hour incubation with Vero-TMPRSS2 cells. The mixture of cysteamine HCl and medium was removed, and the cells were cultured in fresh medium for 72 hours. As shown in Figure 1A, no significant toxicity was noted below 20mM cysteamine HCl while some toxicity was noted with 50 mM concentration. We also wanted to determine if prolonged incubation of cysteamine HCl and Vero-TMPRSS2 as done for the plaque assay would induce any cytotoxicity in the target cells. Different concentrations of cysteamine HCl were pre-incubated with medium for 2 hours followed by incubation with Vero-TMPRSS2 cells for one hour. The culture medium was then diluted fivefold as done for the plaque assay and the cytotoxicity was measured after 72 hours. While initial incubation of cells with 50 mM cysteamine HCl did induce cytotoxicity, no such effect was observed at lower concentrations of cysteamine HCl (Figure 1B).

Before performing the inhibition assay, we wanted to determine the stability of free sulfhydryl (-SH) during the incubation, because free thiols rapidly oxidize in tissue culture medium. The concentration of free sulfhydryl (-SH) in the culture was measured by Elman's reagent and the results are shown in Figure 2. Clearly, no appreciable change in the concentration of free sulfhydryl group was detected both during the pre-incubation of cysteamine HCl with virus for two hours and during the infection step when the virus/cysteamine HCl mixture was incubated with Vero-TMPRSS2 cells for an additional hour. However, a sharp drop in the concentration of -SH group was noted after 24 hours and 72 hours of incubation.

Inhibition of SARS-CoV-2 variants by cysteamine HCl

To determine dose dependent inhibition of SARS-CoV-2 by cysteamine HCl, Vero-TMPRSS2 was selected as this cell line has been shown to be highly permissible to both wild type and the four variants tested in this study. Moreover, these viruses produce defined plaques when assayed in this cell line. Dose dependent inhibition of wild type as well as alpha, beta, and gamma variants are shown in Figure 3A. As described in the Materials and Method section, SARS-CoV-2 was pre-incubated with different concentrations of cysteamine HCl at 37°C in 5% CO2 for 120 min and the mixture was added to Vero-TMPRSS2 cells. After one hour of infection the plates were overlaid with 1.5% of methyl cellulose overlay and the plaques were developed and scored after 72 hours. For controls virus was treated the same way with medium instead of cysteamine HCl. For positive control anti-RBD rabbit polyclonal IgG was used and assayed in an identical manner. As shown in Figure 3, both wild type WA strain as well as the alpha, beta and gamma variants were clearly inhibited in a dose dependent manner compared to the control infection with IC50 values ranging from 1.252 mM for wild type to 1.528 mM for alpha, 0.8 mM for beta, and 1.976 mM for gamma variant. Similar dose dependent inhibition of infection was also noted when the delta variant was treated with different concentrations of cysteamine HCl (Figure 4A) with an IC50 value of 1.066 mM. The delta variant was markedly inhibited by 10 mM cysteamine HCl after 120 min of pre-incubation followed by 60 min of infection of Vero-TMPRSS2 cells. In the above assay cysteamine HCl and the virus inoculum were not removed after initiation of infection before addition of methyl cellulose overlay. A similar pattern of inhibition of delta virus was noted when cysteamine HCl was removed after one hour of infection of Vero-TMPRSS2 cells (data not shown) although a slight increase of IC50 value was noted. Therefore, it is likely that the inhibition of virus infectivity might have occurred during the first three hours of infection when there was high concentration of free thiol group present in the culture to reduce the cys-cys bond of RBD thereby inactivating the virus (Figure 2). Anti-rabbit RBD polyclonal IgG inhibited infection of all viruses in a dose dependent manner (data not shown).

Once the anti-SARS-CoV-2 activity of cysteamine HCl was established we wanted to determine if time of incubation of virus with cysteamine HCl would affect antiviral activity. Virus was pre-incubated with either 5 or 10 mM of cysteamine HCl for 0, 15, 30, 60 and 90 minutes. The mixture was then added to Vero-TMPRSS2 cells and infection continued for an additional 60 min resulting for the total incubation time of virus and cysteamine HCl to be 60, 75, 90, 120 and 150 minutes. As shown in Figure 4B, inhibition of delta variant was noted post incubation with both 5 and 10 mM cysteamine HCl although the effect was more pronounced when the virus and cysteamine HCl was incubated for 150 minutes. Level of inhibition of infection noted here was slightly less than that observed in Figure 4A presumably because the association time of virus with cysteamine HCl in this assay was 150 min when assayed after 90 min pre-incubation compared to 180 min in Figure 4A.

Discussion

In this study the antiviral activity of cysteamine HCl was tested against wild type and variants of SARS-CoV-2 that have emerged so far including the delta variant which has spread in various regions of the world with devastating consequences. For this assay Vero-E6 cells over-expressing the transmembrane serine protease II (Vero-TMPRSS2) were used as this cell line is highly susceptible to infection with wild type and all variants. Our results demonstrate *in vitro* antiviral activity of cysteamine against both wild type and multiple variants of SARS-CoV-2. Similar inhibition of the infectivity of SARS-CoV-2 with cysteamine was also recently demonstrated where the IC50 values were shown to be lower than what was noted in our study (16). This difference is most likely due to the highly susceptible target cells used in our study compared to the Vero E6 cells used in the previous work. The spike protein of SARS-CoV-2 has 13 disulfide bonds (16). It is likely that cysteamine reduces at least some of the disulfide bonds leading to altered conformation, which in turn, inhibits the binding of the receptor binding domain (RBD) of S1 protein spike protein with the ACE-2 receptor on the target cells (17, 18). Since disulfide residues in the RBD are highly conserved among the emergent variants, it is not surprising that all four variants were sensitive to inhibition by

cysteamine HCl. Besides SARS-CoV-2, cysteamine was also shown to have antiviral activity against HIV-1. Envelope protein gp120, which is essential for viral entry,has 10 disulfide bonds (12). Cysteamine reduces these bonds, potentially altering the tertiary protein structure and impeding gp120 binding to CD4 lymphocytes and thus decreasing the cytopathic effect (13,14). Cysteamine has also been shown to inhibit HIV replication (15).

Cysteamine is a free thiol with the odor and taste of rotten eggs, accounting for the olfactory and taste aversion. Despite this, cysteamine has been shown to be well tolerated when administered in humans. Side effects are primarily limited to nausea, vomiting and gastric hyperacidity from the oral preparations. This limits compliance in some younger patients on chronic oral cysteamine therapy, however 94% of patients > 11 years of age reported always being compliant, compared to 50% < 11 years of age who were less compliant (19). Cysteamine for chronic treatment of cystinosis employs a ramp up from an initial 10 mg/kg/d in 4 divided doses to 50 mg/kg/d employing 10 mg/kg/d dose increments at two-week intervals. Prior to the dose escalation protocol side effects seen in 3 patients included hyperthermia, lethargy, and rash (20). Cysteamine eye drops , used to treat the crystalline keratopathy of cystinosis, are very well tolerated and are marketed in both immediate and delayed release forms. The first ocular form to be FDA approved, Cystaran, is recommended to be given hourly while awake. A more recent, long-acting form, Cystadrops, is administered four times a day while awake. Both are well tolerated but may provoke burning and mild eye irritation is some patients. Both require lifelong treatment (21).

Because of the minor side effects observed following chronic cysteamine treatment and the fact that cysteamine also inhibits infection of SARS-CoV-2 variants , the possibility of treatment of COVID patients with cysteamine exists. It is generally accepted that nasal epithelium cells located in the nasopharynx are: (i) the initial source of individual infection with SARSCoV2; (ii) the location of rapid replication and mutation of the virus, and (iii) the primary source from which the virus spreads to others. The nasal epithelium is a target for SARSCoV-2 to enter and replicate via the concentrated

ACE2 receptors on goblet cells (22). As the virus proliferates in the nasal epithelium, lysis of epithelial cells releases virions in a logarithmic progression which then transit to the trachea, bronchi and ultimately alveoli, leading to pneumonia, devastating illness (23), and markedly greater infectivity, as measured by R_{RI} (24). Since cysteamine is currently marketed as ophthalmic preparations, Cystaran ® (0.44%,= 39 mM), and Cystadrops® (0.37%, = 33mM) the drug could be administered topically to the nasal epithelium in concentrations of 1/3 to 1/4 of that used for life-long frequent ocular administration, and which inactivate the virus in vitro in tissue culture. In that location it could act as a chemical impediment to nasal entry and thus to virus replication. Cysteamine employed as a nasal spray, cream or drops could function as both a preventative and mitigator of COVID 19 infection. The delta variant is believed to result in significantly higher viral loads in the nasopharynx than the original virus, thus providing significantly greater opportunities for viral spread and mutation. One method would be nasal administration of a compound such as cysteamine to disrupt the virus at its point of infection and incubation. Reducing or eliminating the virus's ability to infect nasal epithelium cells would not only reduce its ability to sicken infected individuals, but also reduce or eliminate its ability to replicate, mutate and infect others.

Utilization of a nasal spray as a means of administering an antiviral agent to prevent or mitigate SARSCoV-2 infection requires exposure of the virus to the antiviral for a period sufficient to inactivate the virus. The terminal velocity of a particle in a viscous fluid can be determined by balancing the buoyancy force with the drag force obtained using Stokes' law (25). Using the known diameter (100nm) and mass (10-18 kg) (26) of the virus, the terminal velocity in water @ 25°C is found to be 5.6 x 10^{-9} m/s. This assumes, of course, that the virus is a perfect sphere, which it is not. The spike proteins will increase the resistance; therefore, this is an over-estimate of terminal velocity. The nasal surface area is reported at 160 cm2 (27,28). Assuming a nasal spray of 0.5 ml in each nostril, a volume well tolerated by children (29), delivery of 1 ml total volume to the nasal surface would yield a uniform thickness of the antiviral solution of 1 cm3 / 160 cm2 = .0063 cm, or 63 µ (630 virion

diameters) . This would be traversed by a virion at terminal velocity in 1.125 x104 sec, or ~ 3 hours, 1 hour greater than the time required to permit 100% viral inactivation in 10mM cysteamine in tissue culture. This assumes both uniform distribution of the nasal spray throughout the nasal epithelium, and also uniform distribution of ACE2 receptors, both of which are probably incorrect, but not reported at this time. Nasal administration of pharmacologic agents is becoming increasingly favored, although most are used to deliver systemic agents or to target the CNS. As of this writing there are 3645 nasal administration results in PubMed. Employing nasal spray is a potential advantage in smaller children who are averse to masks, or whose parents are opposed to vaccines.

Summary

Results presented in this communication demonstrate significant inhibition of infection of several variants of SARS-CoV-2 including the delta variant with cysteamine HCl in a highly permissible cell line Vero-TMPRSS2. Cysteamine is a well-studied drug with very good safety profile in chronic oral and ocular topical treatment of patients with cystinosis. Potential application of cysteamine may include topical administration of cysteamine to the nasal mucosa of exposed and infected individuals. Such treatment could be used at concentrations of 1/3 to 1/4 that well tolerated in the FDA-approved eyedrops used lifelong.

Jess Thoene and Robert Gavin are inventors on a patent application related to the use of cysteamine for the prevention and treatment of COVID 19 infections. It is assigned to the University of Michigan, licensed by ACIC Pharmaceuticals , Inc who funded this study.

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Figure Legends

Figure 1: Cytotoxicity of cysteamine HCl in Vero-TMPRSS2 cells. Cysteamine HCl was preincubated with Dulbecco's modified Eagle´s medium (DMEM) supplemented with 2% FBS, L-glutamine, 1% penicillin/streptomycin and puromycin (10 µg/ml) for 2 hours at 37°C in 5% CO2 in 100 µl medium and then transferred to the wells containing Vero-TMPRSS2 cells. After 1 hour of incubation medium from each well was removed and 100 µl of complete DMEM medium containing either no cysteamine-HCl (A) or 20% of the original concentration of cysteamine-HCl was added to mimic the condition of the infection assay method (B). Cells were cultured for 72 hours at 37°C in 5% CO2 and cytotoxicity measured as described in the Method section.

Figure 2: Concentration of free sulfhydryl present in the culture: Four concentrations of cysteamine HCl were used, and free thiol content was measured at the initiation of pre-incubation with virus (0 hour), after pre-incubation with virus (2 hour), during incubation of cysteamine HCl/virus mixture with Vero-TMPRSS2 cells (3 hour), 24 and 72 hours post infection as described in the Method Section.

Figure 3: Dose dependent inhibition of infection of wild type and SARS-CoV-2 variants by cysteamine HCl. Wild type (25 pfu/well), alpha (125 pfu/well), beta (18.75 pfu/well) or gamma (25 pfu/well) were preincubated with different concentrations of cysteamine HCl at 37°C for 2 hours in a total volume of 600 µl of complete DMEM medium. Cysteamine HCl/virus mixture was then transferred to each well of Vero-TMPRSS2 cells in a total volume of 250 µl and incubated for 1 hour at 37°C in 5% CO2. Each well was then overlaid with 1 ml of culture medium containing 0.5% methylcellulose and incubated for 3 days at 37°C in 5% CO2 and plaques were developed and scored as described in the Method section. Mean percent inhibition of infection compared to the untreated control is plotted.

Figure 4 Inhibition of delta variant infection by cysteamine HCl.

(A) Delta variant of SARS-CoV-2 (37.5 pfu/well) was preincubated with different concentrations of cysteamine HCl at 37°C for 2 hours in a total volume of 600 µl of complete DMEM medium. Cysteamine HCl/virus mixture was then transferred to each well of Vero-TMPRSS2 cells in a total volume of 250 µl and incubated for 1 hour at 37°C in 5% CO2. Each well was then either overlaid with 1 ml of culture medium containing 0.5% methylcellulose and incubated for 3 days at 37°C in 5% CO2. . (B) Delta variant (37.5 pfu/ml) was preincubated with cysteamine HCl (5 or 10 mM) for 0, 15, 30, 60 and 90 min in a total volume of 600 µl of complete DMEM medium. Cysteamine HCl/virus mixture was then transferred to each well of Vero-TMPRSS2 cells in a total volume of 250 µl and incubated for 1 hour at 37°C in 5% CO2. Each well was then overlaid with 1 ml of culture medium containing 0.5% methylcellulose and plaques were developed as described in the Method section. In both assays plaques were developed and scored as described in the Method section. Mean percent inhibition of infection +/- standard error compared to the untreated control is plotted. Total time of association of virus and cysteamine before addition of 0.5% methyl cellulose overlay was plotted.

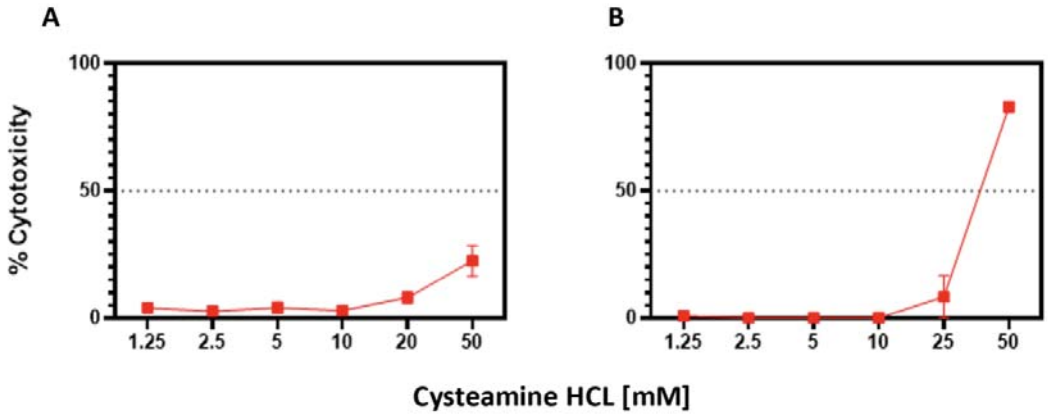

Figure 1

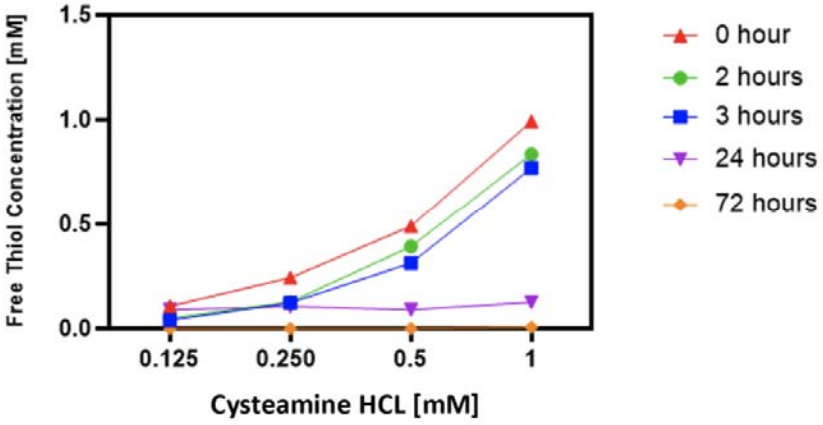

Figure 2

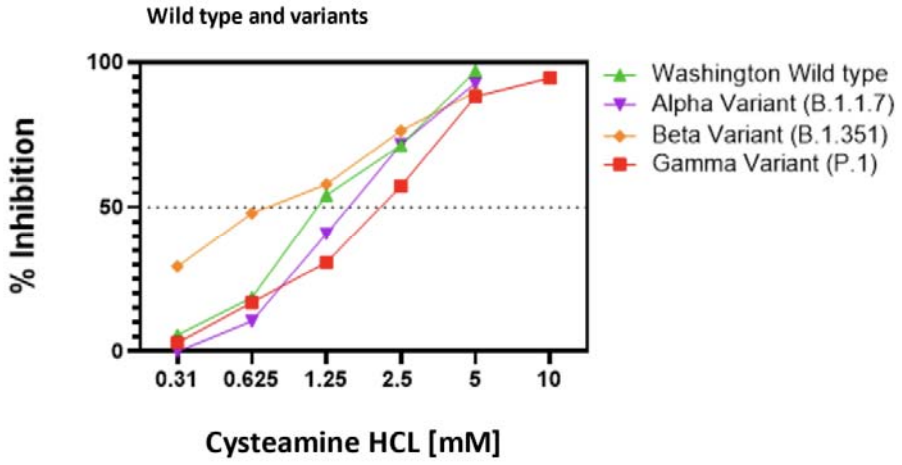

Figure 3

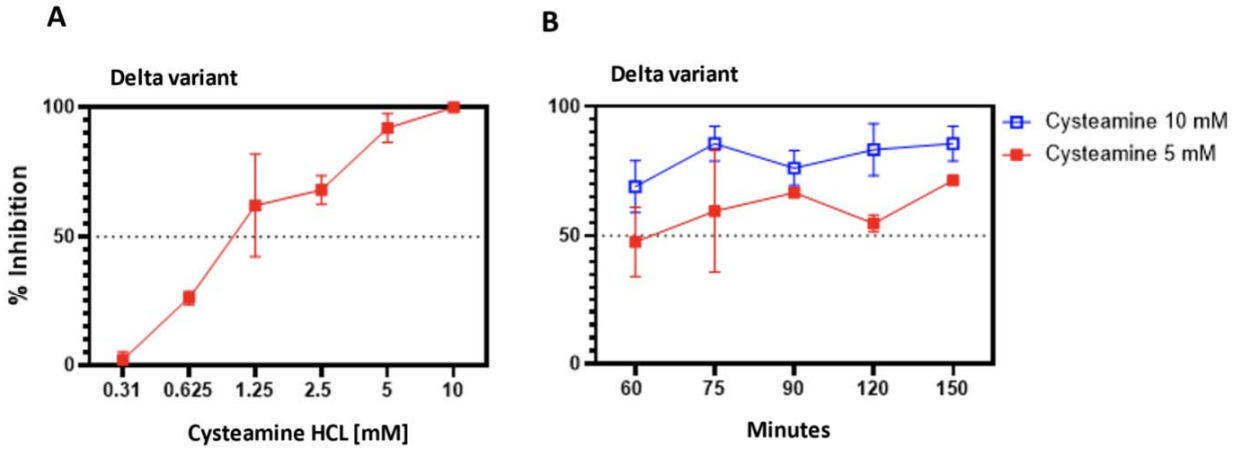

Figure 4