REPORT

FB-1603 for Pseudovirus-SARS-CoV-2 (Alpha strain, Gamma strain, Delta strain) Inhibition in Cellular Model

Testing Article:
FB-1603

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Table of Contents

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Summary

FB-1603 for Pseudovirus-SARS-CoV-2 (Alpha strain, Gamma strain, Delta strain) Inhibition in Cellular Model

FB-1603, which was previously known as *Apogen*® or FE-L-APO, is the API (active pharmaceutical ingredient) of Apomivir®. It is a cold-water extract from microalgae, *Arthrospira* FEM-101 strain. This report is to evaluate its efficacy against SARS-CoV-2 through Pseudovirus-SARS-CoV-2 infection in 293T cells transfected with human ACE-2 receptor protein.

The current study used lentiviral packaging system to package the SARS-CoV-2 Spike protein and a luciferase reporter gene into a pseudovirus. Our previous study on FB-1603 has found that it could inhibit other human viruses such as enteroviruses, e.g. EV-A71 and coxsackievirus A6, and coronavirus 229E, infection in the susceptible cells. To further our understanding about FB-1603 against other coronaviruses, we aim to evaluate the inhibitory effect of FB-1603 on SARS-CoV-2 infection. The result shows FB-1603 can suppress three variants of concern (VOC) of SARS-CoV-2 from infecting ACE2-expressing 293T cells. FB-1603 can inhibit 50% Alpha strain from infection at concentration of 138.2 μg/mL; suppress 50% Gamma strain at 85.1 μg/mL; and repress the most contagious Delta strain at 77.9 μg/mL.

Introduction

SARS-CoV-2, the virus results in the disease COVID-19, was firstly discovered in Wuhan, China and soon after it was found that SARS-CoV-2 can spread from human to human. Since then, the virus has swept across the world with different variants arising at random due to the virus nature to mutate. The number of people infected by the disease continues to change every day. While the impact of the disease varies by location, there are more than 218.5 million confirmed cases of people with COVID-19 around the globe and more than 4.5 million people have died from the disease, according to the WHO.

According to the CDC, reported COVID-19 illnesses have ranged from mild to severe to the point of requiring hospitalization, intensive care, and/or a ventilator. COVID-19 illnesses can also lead to death. While people of all ages can be infected, the risk for complications increases with age. People living in a nursing home or long-term care facility, and people of all ages with underlying health conditions (such as diabetes, heart disease, lung disease, and obesity) also are at high risk for serious illness. COVID-19 also has led to serious illness and even death in younger and middle-aged adults who are otherwise healthy. While most children have mild or no symptoms, some have gotten severely ill. As with adults, even if children have no symptoms, they can spread the virus to others.

Infection prevention is key to slow the spread of the disease. Near the beginning of the pandemic, public health experts directed their efforts toward "flattening the curve." It means fewer patients during the patient surge period, and hospitals would be better able to manage the demands of patients who are sick with COVID-19 and other illnesses. With the major vaccine developments of Pfizer/BNT, Moderna and AZ, Johnson & Johnson, governments around the world hope to control COVID-19 pandemic through herd immunity. However, capped vaccine production and never-ending needs for them across the globe, mean that there will always be a delay between vaccine shots. Certainly, other preventive measures are being researched and developed, and our company, Far East Bio-Tec Co., Ltd. has long invested in developing antiviral ingredient from algae. We have found the Spirulina extract has antiviral activity and further refined the ingredient to FB-1603.

FB-1603, previously known as *Apogen*® or FE-L-APO, is the API (active pharmaceutical

ingredient) of Apomivir®. It is a cold-water extract from microalgae, *Arthrospira* FEM-101 strain. This report is to evaluate their efficacy of SARS-CoV-2 inhibition through pseudovirus infection in transfected 293T cells expressing human ACE-2 receptor protein. We used lentiviral packaging system to package the SARS-CoV-2 Spike protein and a luciferase reporter gene into a pseudovirus. With only SARS-CoV-2 Spike protein present in the virus, the model is suitable for screening inhibitors targeting Spike-ACE2 interaction. Previously we found FB-1603 can inhibit other human viruses like enteroviruses, e.g. EV-A71 and coxsackievirus A6, infection in the susceptible cells. To further our understanding about FB-1603 against coronaviruses, we aim to evaluate the inhibitory effect of FB-1603 on SARS-CoV-2 infection. Prior work has indicated that FB-1603 can suppresses the WT strain of SARS-CoV-2 (Wuhan strain) from infecting ACE2-overexpressing 293T. We hope to expand the study to investigate whether FB-1603 has inhibitory effect on different SARS-CoV-2 variants. All viruses, including SARS-CoV-2, the virus that causes COVID-19, change over time. Most changes have little to no impact on the virus' properties. However, some changes may affect the virus's properties, such as how easily it spreads, the associated disease severity, or the performance of vaccines, therapeutic medicines, diagnostic tools, or other public health and social measures. There have been several SARS-CoV-2 variants discovered and the mutations found are usually at the spike protein, which is the primary target for immune surveillance. In the current study, we tested Alpha, Delta, and Gamma strains of concern.

Alpha strain (Pango lineage B.1.1.7) was first identified in UK and was the first major variant spreading faster than the original Wuhan-CoV and soon replaced it to become the dominant SARS-CoV-2 strain during the summer in 2020. Current vaccines work well against alpha strain with limited cases of breakthrough infection and convalescent and post-vaccination sera also neutralize alpha strain efficiently. Gamma strain (Pango lineage P.1) was first discovered in Japan and Brazil. Although vaccine-induced immunity work well against gamma strain, certain monoclonal antibody treatments has reduced neutralization ability against it. Delta strain (Pango lineage B.1.617.2) was first identified in India and is now the most aggressive variant spreading the whole world. While there are more reported breakthrough infections with delta strain compared with others, vaccines do protect people from hospitalization with delta strain.

In this report, we aimed to examine FB-1603 ability to suppress the interaction between SARS-CoV-2 spike protein and the human cell ACE2 receptor in cellular model.

Materials and Methods

1. FB-1603 preparation

The testing article, FB-1603 supplied by Far East Bio-Tec Co., Ltd. was dried by frozen-vacuum and stored in 4°C refrigerator. At the time of using, weigh appropriate amount of FB-1603 powder and resuspended with autoclaved PBS. Information regarding the methods of synthesis, composition and other characteristics that defines the test article are on file with Far East Bio-Tec Co., Ltd.

2. Pseudovirus-SARS-CoV-2 preparation

Three variants of concern (VOC) of SARS-CoV-2 pseudovirus B.1.1.7 (alpha strain), Lineage P1 (gamma strain), and B.1.617.2 (delta strain) were purchased from RNAi Core of Academia Sinica, Taiwan. Briefly, the pseudoviruses are prepared by using a VSV pseudotyped virus packaging system, and contains the spike protein (S protein) from SARS-CoV-2 while the VSV glycoprotein (VSV G) is substituted with luciferase gene reporter, thus the virus can only infect cell once and the infected cells will express luciferase.

3. Cell culture and infection

293T cell transfected with human ACE-2 gene was obtained from Dr. Shih's lab (RCEVI, CGU). 293T-ACE2 was cultured in complete DMEM. On the day before virus infection, 96-well plates were first coated with Poly-L-Lysine (5 times dilution, Sigma) for a hour, followed by seeding 293T-ACE2 at 4×10^4 cell/well. On the day of infection, dilute FB-1603 with DMEM containing 2% FBS to desired concentration and mix with 4 μl nCoV-Luc/well. The final volume of drug+virus is 240 μl. Remove medium from 293T-ACE2 plate; add 200 μl drug+virus mixture to each well. Culture the infection plates in 37°C humidified incubator with 5% CO_2. 48 hours later, remove medium from all wells and add 100 μl Bright Glo (Promega) diluted half and half with serum-free DMEM, mix well and transfer to white plates for luminescence detection. Values from each well first subtracts baseline from non-infected cells, and compared with virus infection control to obtain the infectivity percentage under each drug concentration.

Results and Discussion

FB-1603 is an efficient inhibitor of SARS-CoV-2 infection according to this pseudovirus study. The result shows FB-1603 can suppress 50% of Alpha strain infection at 138.2 μg/mL, 50% of Gamma strain at 85.1 μg/mL, and 50% of Delta strain at 77.9 μg/mL.

Comparing with Remdesivir, an adenosine analogue, EC50 of Remdesivir is 0.77 μM. The inhibitory mechanism of remdesivir is through incorporation into nascent viral RNA strands which results in premature termination. However, Remdesivir through clinical trials was found to be more effective in Caucasians and people with mild symptoms, while Asians and patients with advanced disease progression received little to no therapeutic effect. In addition, liver failure is a common side effect for Remdesivir treatment. Microalgae extracts, on the other hand, has a long history as food supplement and Far East Bio-Tec Co., Ltd. has developed several nutrient supplement products based on Arthrospira maxima. The history of microalgae as food proves its safety aspects in humans.

In summary, our findings reveal that FB-1603 is effective against the interaction between SARS-CoV-2 spike protein and the human receptor ACE-2. FB-1603 has a great potential for prevention for evaluation as preventive measures against SARS-CoV-2 infection.

Conclusion

FB-1603 can inhibit three popular variants of SARS-CoV-2 infection through interference of virus-cell interaction. The result demonstrates the efficacy of FB-1603 and proves it can be a promising agent for SARS-CoV-2 intervention and prevention.

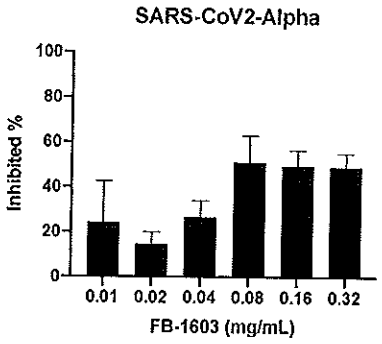
Reference

Chen, Y. H., et al. (2016). "Well-tolerated Spirulina extract inhibits influenza virus replication and reduces virus-induced mortality." Sci Rep 6: 24253

Wang, M., *et al.* Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* **30,** 269–271 (2020).

Figures and Tables

a.



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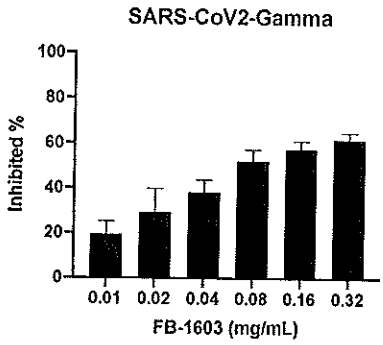

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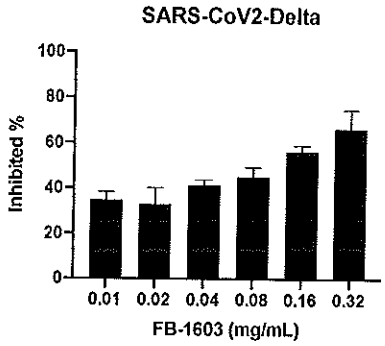
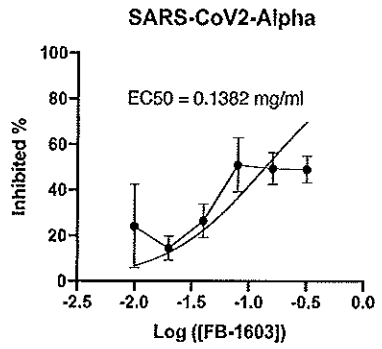


Figure 1. FB-1603 at various concentration and percentage of inhibition.

293T-ACE2 cells were infected with 3 variants of pseudotyped and treated with FB-1603 at the same time. The treatments were diluted by half from the prior concentration. 48 hours after initial infection, luciferease activity was measured by luminomitor. (a) Alpha strain (B.1.1.7), (b) Gamma strain (P1), (c) Delta strain (B.1.617.1).

a.



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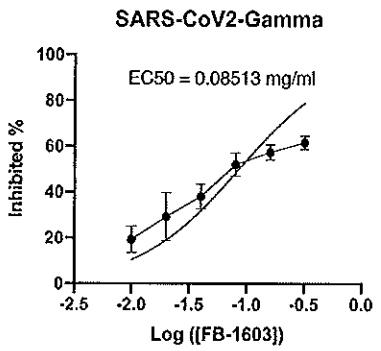

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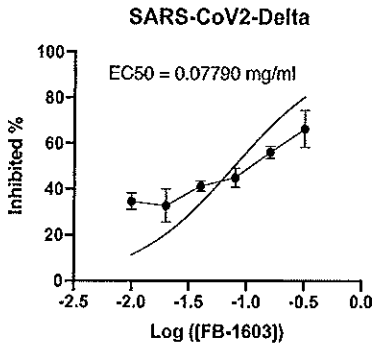

Figure 2. Plotting log(concentration) against pseudovirus inhibition rate (represented by luminescence intensity) to calculate EC50 of FB-1603.

The graph is transformed by taking log of drug concentration and plot against the luminescence intensity from infected cells. EC50 of FB-1603 targeting (a) Alpha strain (B.1.1.7), (b) Gamma strain (P1), (c) Delta strain (B.1.617.1) were calculated with GraphPad 9 by plotting non-linear regression.