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Chang Gung University

Chang Gung University Project Report

Project Title:

Evaluation of Virus-Bom Antiviral Effects on Coronavirus

Project Number:

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I. Introduction

Coronaviruses exist in many animals. In the past, several coronaviruses (human coronavirus) infected humans and caused the common cold or pneumonia. This virus has an envelope structure, the virus particle size of about 120 nm, which contains a positive strand of RNA estimated about 30,000 nucleotides. As the virus spike membrane is embedded in the viral coat, it resembles a crown or a sun's corona-shaped under the electron micrograph, hence it is named coronavirus.

Before the outbreak of SARS (Severe Acute Respiratory Syndrome), human coronavirus did not attract public attention. However, since the end of Year 2002- 2003, the SARS epidemic broke out in China, and cases started to show up in Taiwan at the beginning of March, 2003. Due to seriousness of the disease and the rate of fatality is high (about 10%), SARS caused great panic in Taiwan. Later, scientific research found that the causative agent of SARS was coronavirus, and it was named SARS-CoV. Current research suggests and indicates that the natural host of the virus is bats, which may spread to humans through the intermediate host civet cat. Nine years after the end of the SARS epidemic (2012), scientists discovered that another coronavirus is causing serious disease in humans - the Middle East Respiratory Syndrome (MERS) coronavirus infection. A total of about 2,500 people had been diagnosed in this infectious disease in the world by September 2019, with an infection mortality rate of 34%. Therefore, MERS-CoV is a highly pathogenic coronavirus. Current research suggests that the earliest source of MERS-CoV is still bats, which spread to humans through camels as intermediate hosts. While the MERS-CoV epidemic was under control, at the end of 2019, an unexplained pneumonia broke out in Wuhan, China, and it was later proved that this pneumonia was caused by a new type of coronavirus, which was named 2019 novel coronavirus (2019-nCoV), and later The Virus Nomenclature Committee (ICTV) named it SARS-CoV-2, and WHO named the disease caused by this virus "Coronavirus Disease-2019," referred to as COVID-19. In just 2-3 months (until February 21, 2020), there have been more than 70,000 confirmed cases and more than 2,000 deaths worldwide. According to the latest research, the source of this virus may still be bats, but the intermediate host has not yet been determined.

The purpose of this study is to use established quantifiable coronavirus strain (229E) techniques, such as plaque reduction assay (plaque reduction assay), to evaluate whether Virus-Bom can eliminate coronavirus 229E and inhibit viral activity ability. Expecting and hoping to develop sustainable products with effective anti-viral capabilities, which will be helpful to virus transmission and prevention.

II. The purpose of the design

To investigate whether the product, Virus-Bom, has the effectiveness of killing coronavirus 229E, to also test and verify whether it has the efficacy and quality.

III. Research methods and implementation steps

a. Experimental materials and equipment

Test material name: Virus-Bom

Cell line — Huh 7 cell (human liver cell line)	
Virus strain — Coronavirus 229E	
Cell culture — Dulbecco's Modified Eagle Medium	Gibco
Fetal bovine serum	Gibco
Non-essential amino acid	Gibco
Antibiotic-antimycotic	Gibco
Sodium bicarbonate	J.T. Barker
Sodium phosphate	Sigma
Trypsin-EDTA (2.5%)	Gibco
Plaque assay — Agarose	Amersco
(Neutralization test) Crystal violet	J.T. Barker
Formalin	Riedel-deHAen

b. Experimental process

1. Cell culture

The cells were cultured in 10% FBS DMEM medium and placed in a 37°C incubator that containing 5% CO2. When the cells are subcultured, we first wash the cells twice with PBS, and then add the appropriate amount of trypsine-EDTA to treat the cells. After the cells are shed from the surface of the culture dish, we then add 10% FBS DMEM culture medium to evenly disperse the cells and place them in the culture dish. Incubate in a 37°C incubator containing 5% CO2.

2. Virus amplify

Coronavirus 229E is cultured with Huh7 cells, and the cells are cultured in 10% FBS DMEM culture medium. When they grow to about 90%, we washed with PBS and use the 0.01 multiplicity of infection (MOI) to remove the infected cells. And add 0%

FBS DMEM culture medium, place in a 5% CO2 incubator at 35 ℃ for about 48 hours. When cytopathic effect (CPE) occurs in 50% of cells, all cultures containing virus and CPE cells are collected, centrifuged at 2000 rpm for 10 minutes, and all supernatants are collected and stored in refrigerators at -80°C.

3. Antiviral assay

i. Cell preparation

After the cells were release with trypsin-EDTA, the cell concentration was adjusted to 6 x 10^5 cells/ml with 10% FBS in DMEM culture medium, 1 mL was inoculated into a 6-well plate, and placed in a 37˚C incubator with 5% CO2 Cultivate for 18~24 hours as backups.

ii. Virus and analyte interaction

Virus-Bom was dissolved in an aqueous ethanol solution (ethanol: water = 1:10) at concentrations of 100 ppm, 300 ppm, and 500 ppm. This solution was mixed with the above virus-containing cell culture solution in an equal amount of 9:1, and allowed to stand at room temperature (about 25°C) for 30 minutes. This is a virus processing group. The same concentration of Virus-Bom and the same amount of cell culture fluid without virus addition were used to perform the same effect as described above to serve as a control group.

iii. Antiviral assay

In the 6-well plate that has been added to the cell, we add the Virus-Bom that has interacted with the virus to disintegrate it, and use it as a test substance. Dilute the test substance in a 10-fold sequence to the 10th to the 8th power, and then put it back into the incubator for cultivation for 48-64 hours. Lastly, cells were fixed with 10% formalin for 1 hour, and then stained with 0.1% crystal violet for 5 minutes. We then count the number of virus spots compared to the control group to know the anti-virus ability of the test sample.

iv. Data and results:

After staining with 0.1% crystal violet, and using the formula to calculate the effect of virus spot inhibition. The result of the case report must present a graph of the inhibitory effect of Virus-Bom on Coronavirus 229E.

IV. Research results

The Virus-Bom was dissolved in an aqueous ethanol solution (ethanol: water=1:10) at concentrations of 100 ppm, 300 ppm, and 500 ppm, respectively. This solution was mixed with the above virus-containing cell culture solution in an equal amount of 9:1, and allowed to stand at room temperature (about 25°C) for 30 minutes. This is a virus processing group. The same concentration of Virus-Bom was performed as the control group with the same amount of cell culture fluid at the same concentration as that of the virus without adding virus at twice the concentration. Experimental results show that 100 ppm, 300 ppm, and 500 ppm of Virus-Bom can inhibit the virus's ability to generate virus plaque forming, and the effect of inhibiting virus is >99.99 %. Based on the above experimental results of inhibiting coronavirus 229E, Virus-Bom has the effect of inhibiting coronavirus 229E.

V. chart

Table 1. The results of Virus-Bom inhibiting coronavirus 229E

	Viral inhibition rate (%)		
	Test 1	Test 2	Test 3
500ppm	>99.99%	>99.99%	>99.99%
300ppm	>99.99%	>99.99%	>99.99%
100ppm	>99.99%	>99.99%	>99.99%

We put the VirusBom product by Money Marketing Communication Ltd. and Coronavirus 229E at room temperature according to the standard laboratory operating time. After 30 minutes of reaction, the liquid was added to the cells to observe the virus's ability to generate virus plaque forming. The higher the inhibition rate (%), the better the ability to inhibit viruses.

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