The Effects of Chinese Medicine Lianhua Qingwen on Inhibiting the Replication of Covid-19 Virus

Hongzhu Ji
Fordham University FCLC, New York, U.S.A.

Keywords: Chinese Medicine, Lianhua Qingwen, Covid-19, Virus Replication.

Abstract: Lianhua Qingwen, a commonly used traditional Chinese medicine, has been used to treat fever and respiratory infections for many years including SARS. Recent studies have shown that it can also be used as a COVID-19 treatment. The goal of this work is to test if Lianhua Qingwen fights against coronavirus by reducing virus replication? The antiviral activity of Lianhua Qingwen against SARS-CoV-2 was assessed in infected cells from patients using CPE assay and plaque reduction assay. There are six possible results: they are based on if Lianhua Qingwen shows positive, no, or negative inhibition on the CPE inhibition assay and weather it gives us an inhibitory effect on the formation of plaque of the virus or not, and we will say Lianhua Qingwen significantly inhibited SARS-CoV-2 replication in cells which partially supports our hypothesis, or it partly supports our hypothesis and these results wouldn't give a clear answer to our research question, or it contradicts our hypothesis. The result of our study will provide important information for how Lianhua Qingwen helps to treat COVID-19, and it will help us with controlling the COVID-19 pandemic. Future studies should focus on more potential effects of Lianhua Qingwen and vivo experiment is needed.

1 INTRODUCTION

COVID-19 is a highly contagious respiratory infectious disease caused by severe acute respiratory syndrome coronavirus - 2 (SARS - CoV - 2)(Xia et al. 2020). It's an animal transmission disease that is likely originated from bats and spread dramatically in humans, and now it has widely spread around the world. By now there are more than 115 million cases and 2.55 million deaths. This COVID-19 pandemic not only severely damaged people's life and health, but also harmed social, economic, and political. The symptoms of it are fever, dyspnea, nasal congestion, sore throat, asthenia, dry cough, multiple organ dysfunction, and death. China has successfully controlled the pandemic with its strict policy and traditional Chinese medicine including Lianhua Qingwen.

Additionally, by analyzing COVID-19 infected patients' clinical records from Wuhan Ninth Hospital and CR & WISCO General Hospital, researchers found the Lianhua Qingwen combination could allay fundamental symptoms significantly and shorten the progress and development of it (Li et al. 2020). Lianhua Qingwen, the classic traditional Chinese Medicine, is still commonly used today as a treatment for inflammatory fever and respiratory infectious diseases. It was used to fight against SARS in China from 2002 to 2003, and it was included in the Guideline for the Diagnosis and Treatment of Novel Coronavirus (2019-nCoV) Pneumonia (On Trials, the Fourth/Fifth/ Sixth/Seventh Edition) issued by the Chinese government. Lianhua Qingwen consists a total of 13 different herbs: Lian Qiao, 255 g; Ma Huang, 85 g; Jin Yin Hua, 255 g; Ban Lan Gen, 255 g; Mianma Guanzhong, 255 g; Bo He, 7.5 g; Shi Gao, 255 g; Guang Huo Xiang, 85 g; Hong Jing Tian, 85 g; Yu Xing Cao, 255 g; Da Huang, 51 g; Ku Xing Ren, 85 g; and Gan Cao, 85 g (Hu 2020). Previous studies had shown using conventional drugs with the addition of Lianhua Qingwen is a treatment with bright potential for both pneumonia and the COVID-19 virus, and by using Lianhua Qingwen the healing period of COVID-19 is shortened and several symptoms like fever, cough, fatigue, sputum, muscle aches, difficulty breathing, chest tightness and pulmonary imaging can be improved (Hu et al. 2020). These studies have shown that Lianhua Qingwen relieved symptoms of the COVID-19 remarkably, but research about the fundamental mechanism is elusive.

Ji, H.
The Effects of Chinese Medicine Lianhua Qingwen on Inhibiting the Replication of Covid-19 Virus.
DOI: 10.5220/0011292900003438
In *Proceedings of the 1st International Conference on Health Big Data and Intelligent Healthcare (ICHIH 2022)*, pages 284-288
ISBN: 978-989-758-596-8

Therefore, my research question is: Is the use of Lianhua Qingwen block the replication of the COVID-19 virus? And my hypothesis is I predict that Chinese Medicine Lianhua Qingwen helps to block replication of COVID-19. I will treat lung cells from COVID infected human patients with an increasing amount of Lianhua Qingwen (0mg/mL which is the buffer solution, 0.1 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 0.7 mg/mL, and 1 mg/mL) for the various durations (12h, one day, three days, and seven days) and measure it by CPE inhibition assay and plaque assay with 10 replicates.

2 METHOD

2.1 Materials

This experiment will use COVID-19 infected cells (experiment cells) from patients, and the growth of cells was supported at 37 °C by Dulbecco's Modified Eagle's medium (DMEM) along with fetal bovine serum at the concentration of 10 %. The infection experiments were conducted in a biosafety level-3 laboratory for safety concerns. Lianhua Qingwen capsules were obtained and the black powder of it was removed from the capsules. The black powders were dissolved in dimethyl sulfoxide first to 200 mg/mL. Next, at room temperature mix the solution by shake or stir it for 30 minutes, and prepare the stock solution by diluting the Lianhua Qingwen solution with serum-free DMEM to the concentration of 0.1 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 0.7 mg/mL and 1 mg/mL, and at the temperature of −20 °C reserve it. Additionally, preparing Remdesivir solution with a similar method by dissolved in dimethyl sulfoxide and mix to the concentration of 100 mM and stored at −20 °C with the Lianhua Qingwen solution, and the dilution buffer that used in later experiments is prepared by DMEM with 2% fetal bovine serum. Materials like 96-well plates, 0.6 % agar, timer, thermometer, and microscope are also needed in the following experiment.

2.2 Cytopathic Effect (CPE) Inhibition Assay

The cytopathic effect is observable abnormalities such as structural changes in host cells that are caused by the viral invasion, and different viruses infect different types of cells will cause different cytopathic effects. CPEs are important aspects of a viral infection in diagnostics. The CPE assay is used to test if a compound exhibits any antiviral efficacy or not. CPE assay is a cost-effective and time-efficient assay that we used to evaluate test articles' ability to inhibit CPE. Many combinations of cells and viruses can be used to measure interferon activity via CPE assay. And by using the dose-response assay we are able to tell the selected antiviral efficacy range (Britannica 2019).Put experiment cells in monolayers inside separate 96-well plates. To explore the effectiveness against experiment virus of Lianhua Qingwen, in the experiment group, the infected cells were incubated with Lianhua Qingwen solution at various concentrations for different durations (12h, one day, three days, and seven days). Then, the positive control group was developed at the different concentrations of Remdesivir solution mentioned before. As for the negative control group, grow the experiment cells with indicated concentrations of solely buffer solution (which contains 0 mg/mL of Lianhua Qingwen). After various times of incubation in these different solutions, the infected experiment cells will show full CPE under the microscope (100%). Next, the CPE at different percentages in all three groups with different solutions treated cells were recorded. Also, 10 replicates will be done for this experiment, and by using the Reed-Muench method we will be able to calculate IC50 which is the half virus-induced CPE of inhibition concentration for all the experiments and the calculated IC50 will be compared later (Li et al. 2020).

2.3 Plaque Reduction Assay

Plaque assay is a way of measuring virus quantity that is used to test the concentration of virus's dose of infection, and it specifies the quantity of appeared units of plaque in the test sample of viruses. A monolayer of infected cells at different concentrations and covered with a semi-solid medium (we will use agar in this experiment), to forestall the infection of virus from spreading randomly. When a certain virus passes infection to a cell monolayer the infected cell area will create a plaque. The culture will then be stained with a dye, which stains only viable cells. We will use 1% crystal violet in this experiment. As a result, the plaque (the dead cells) will appear unstained against the colored background for researchers to count (Kaufmann, Kabelitz 2002). The SARS-CoV-2 infected cells were put in monolayers and were put in 96-well plates separately. And for all three groups, the plates were all covered with 0.6 % agar, 2% fetal bovine serum. Next, for each of the individual groups, the plate was covered with the different concentrations of either

Lianhua Qingwen (0.1 mg/mL, 0.3 mg/mL, 0.5 mg/mL, 0.7 mg/mL, and 1 mg/mL), Remdesivir, or buffer (0.6 % agar, 2% fetal bovine serum together with either one of these three are all considered as agar overlays). Then incubated all plates with 5% CO2 at 37 °C for different durations (12h, one day, three days, and seven days). Following, removed the agar overlays for each plate. And, fixed the cell monolayer with 10 % formalin, stained with 1% crystal violet. This experiment will be done with 10 replicates. Lastly, the plaques for all groups were counted carefully and photographed (Li et al. 2020).

2.4 Statistical Analysis

The statistical significance of all numerical data gathered through the Reed-Muench method will be analyzed using the student's T-Test on GraphPad Prism® at ($p < 0.05$).

3 RESULT

SARS-CoV-2 infected cells from patients were supported by DMEM as specified in the method part. To conduct an inquiry into the effectiveness of Lianhua Qingwen against the virus, the cells were incubated with Lianhua Qingwen at different concentrations for about 72 h. And the positive and negative control groups followed a similar method with Remdesivir which is the positive control group and buffer solution (DMEM with 2% fetal bovine serum) as the negative control. Calculating the IC50 number for the virus-induced CPE of these three experiments by the Reed-Muench method compared the results to test our hypothesis. For the Cytopathic effect inhibition assay, the positive control experiment with Remdesivir would show positive inhibition and the negative control experiment with the buffer (0% of Lianhua Qingwen solution) would show negative inhibition. Lianhua Qingwen could show positive inhibition, no inhibition, or negative inhibition. If the experiment with Lianhua Qingwen solution shows positive inhibition or no inhibition, we will conduct that Lianhua Qingwen inhibited the tested virus replication in experiment cells (because the negative control will give negative results of virus inhibition) which agrees with our hypothesis. Lianhua Qingwen inhibits SARS-CoV-2 replication significantly in infected cells will be represented as a "+" sign in Table#1. For the Plaque reduction assay, after counting the plaques for all groups Remdesivir would show less plaque which indicates positive inhibition and the negative control experiment with the buffer would show more plaque which shows negative inhibition. And if Lianhua Qingwen inhibit or reduced effects of the plaque appearing of the virus, it will be represented as a "+" sign in Table#1, and it agrees with our hypothesis.

There are six possible results: (1). If Lianhua Qingwen shows positive inhibition on the CPE inhibition assay and it prevents the plaque formation of the virus, we will say Lianhua Qingwen appreciably prevent the COVID-19 virus replication in experiment cells which agree with our hypothesis. (2). If Lianhua Qingwen shows no inhibition on the CPE inhibition assay and it gives us the same effect as the first result, and we also say the replication of the virus in cells was invited by Lianhua Qingwen remarkably which agree with our hypothesis. (3). If Lianhua Qingwen shows negative inhibition on the CPE inhibition assay and inhibitory effect was given to us on the formation of plaque in cells, the results of the CPE and plaque reduction assay contradicted each other, which partly proves our hypothesis and these results wouldn't give a clear answer to our research question. (4). If Lianhua Qingwen shows positive inhibition on the CPE inhibition assay and it gives us no inhibitory effect on plaque formation of the SARS-CoV-2 virus, we will the results of the CPE and plaque reduction assay contradicted each other, which partly proves our hypothesis and these results wouldn't give a clear answer to our research question. (5). If Lianhua Qingwen shows no inhibition on the CPE inhibition assay and it shows no inhibitory effect on plaque formation of the SARS-CoV-2 virus, we say the results of the CPE and plaque reduction assay contradicted each other, which partly supports our hypothesis and these results wouldn't give a clear answer to our research questions. (6). If Lianhua Qingwen shows negative inhibition on the CPE inhibition assay and it gives us no inhibitory effect on plaque development of the virus, which means Lianhua Qingwen did not significantly inhibit the tested virus replication in cells and it disagrees with our hypothesis.

4 DISCUSSION

Previous studies had shown Lianhua Qingwen together with drugs that are currently used is a potential bright treatment for COVID-19, and by using it the healing period of COVID-19 is shortened and improves several symptoms. Research has also shown Lianhua Qingwen inhibits virus replication in both Vero E6 and Huh-7 cells, and it has a dose-dependent effect of inhibition on the formation of

plaque. This study will use infected lung cells from patients to verify if Lianhua Qingwen fights against coronavirus by reducing virus replication. Our hypothesis is consistent with previous studies and it predicts that Lianhua Qingwen helps to block replication of COVID-19.

Table 1: All Possible Results.

Antiviral activity of Lianhua Qingwen on SARS-CoV-2 in vitro	Result 1	Result 2	Result 3	Result 4	Result 5	Result 6
Cytopathic effect (CPE) inhibition assay	positive inhibition (+)	no inhibition (+)	negative inhibition (-)	positive inhibition (+)	no inhibition (+)	negative inhibition (-)
Plaque reduction assay	positive reduction (+)	positive reduction (+)	positive reduction (+)	no reduction (-)	no reduction (-)	no reduction (-)
Agree or disagree with the hypothesis	agree	agree	partly agree	partly agree	partly agree	disagree

Possible results one and two are consistent with previous studies investigating Lianhua Qingwen, and they are most likely to happen. Both of these results show inhibition on the CPE inhibition assay and it gives us an negative growth effect on plaque formation of the virus, and they agree with our hypothesis. The difference between these two results is result 1 shows a stronger ability of the inhibition on coronavirus. In result 2, Lianhua Qingwen shows no inhibition on the CPE inhibition assay but it gives us an inhibitory effect on plaque formation. This result is still significant because showing no inhibition still means Lianhua Qingwen succeeded in slowing or even stopping the virus replication. This is essential in controlling the pandemic and it could help save thousands of lives. Based on previous research, between these two results, result 1 is even more likely to happen. These results show how Lianhua Qingwen helps to treat COVID-19 by inhibiting the rapid virus replication, and future studies should focus on more potential effects of Lianhua Qingwen and vivo experiment is needed. Research could also be done about the differences between recovered cells that were treated with different drugs (for example shapes, size, viability, etc.). Also, even these results prove Lianhua Qingwen inhibits virus replication, studies could also find other possible mechanisms that Lianhua Qingwen may use to fight the COVID-19 virus.

Possible results three, four, and five partly prove our hypothesis and these results are controversial and wouldn't give a clear answer to our research question. These results show contradictions between the CPE inhibition assay and the plaque reduction assay, and if there are no errors in operation that caused these contradictions we should design more experiments on the effects of Lianhua Qingwen on virus replication. Future studies should focus on why these two assays give different results and find more ways to test the inhibition effects of Lianhua Qingwen on virus replication. Also, studies that find other possible ways that Lianhua Qingwen could use to fight the COVID-19 virus.

The possible results six contradicts with the current understanding of Lianhua Qingwen's effects on the SARS-CoV-2 virus, this result indicates that Lianhua Qingwen did not inhibit SARS-CoV-2 replication in cells dramatically and it disagrees with our hypothesis. This result is unlikely to happen since there has already been much research that proves relief of clinical symptoms of coronavirus and the inhibition effect that Lianhua Qingwen has on the virus. However, since the mechanism of antiviral effects on coronavirus is new and hard to find, there could be other ways Lianhua Qingwen helps treat coronavirus. Future studies should review the existing researches that are not consistent with this result and focus on finding other mechanisms that Lianhua Qingwen could help fighting coronavirus.

5 CONCLUSIONS

This study explores the effect of Lianhua Qingwen in

the inhibition of the COVID-19 virus. The result of our study will indicate whether or not Lianhua Qingwen fights against coronavirus by reducing virus replication. The possible results that are likely to happen prove our hypothesis that Lianhua Qingwen inhibits virus replication which supports that combines Lianhua Qingwen with existing therapies to help to treat COVID-19 in clinical. The possible controversial results on the infected lung cells from patients will also indicate the inhibition effects of Lianhua Qingwen on coronavirus, and we need to find out what caused these two assays to give us contradicted results. Future studies need to be investigated in clearer details and find other mechanisms that Lianhua Qingwen could help fighting coronavirus and trying to validate this further by conducting vivo experiments.

REFERENCES

Britannica, (2019) The Editors of Encyclopaedia. "Cytopathic effect". Encyclopedia Britannica, https://www.britannica.com/science/cytopathic-effect.

Hu, Caiyun, et al. (2020) Efficacy of Lianhua QINGWEN Compared with Conventional Drugs in the Treatment of COMMON Pneumonia and COVID-19 PNEUMONIA: A Meta-Analysis. www.hindawi.com/journals/ecam/2020/5157089/.

Kaufmann, S.H.; Kabelitz, D. (2002). Methods in Microbiology, Immunology of Infection. Academic Press., ISBN 0-12-521532-0.

Li, Runfeng, et al. (2020) “Lianhuaqingwen Exerts Anti-Viral and Anti-Inflammatory Activity against Novel Coronavirus (SARS-CoV-2).” Pharmacological Research, Published by Elsevier Ltd., www.ncbi.nlm.nih.gov/pmc/articles/PMC7102548/.

Xia, Qi-Dong, et al. (2020) “Network Pharmacology and Molecular Docking Analyses on Lianhua Qingwen Capsule Indicate Akt1 Is a Potential Target to Treat and Prevent COVID-19.” onlinelibrary.wiley.com/doi/full/10.1111/cpr.12949.