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Research Article

Kumazasa Derivatives Showed an New Anti-Virus Activity

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Abstract

Background: A novel coronavirus pneumonia (COVID-19) outbreak in Wuhan, China, was caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), and has now expanded to over 200 countries and territories, infecting more than 2 million people in US and caused over 20 thousand deaths worldwide.

Kumazasa derivatives are reported safety drug to improve anti microorganisms including coronavirus in Japan and its main active ingredient is tricin (4,5,7-trihydroxy-3,5-dimethoxyflavon). The Kumazasa derivatives can suppress the human cytomegalovirus (HCMV) of the DNA virus and the coronavirus pneumonia virus of RNA virus replication *in vitro*.

Materials and Methods: Human embryonic pulmonary fibroblast (HEL), obtained from human fetal lungs cultivated in 9.6 cm^2 shell until confluence with Dulbecco's Modified Eagle Medium (DMEM) medium was carried out, supplemented with 8% amount of fetal bovine serum (FCS). Towne strain of HCMV has been adsorbed and infected in a variety of infections (MOI) = 1 in HEL cells. After incubation at 37°C for 1 hour, concentrations of 0.1, 1μ, 10 μM of tricin or novel compounds were added and cultivated for 6 days, and infectious HCMV numbers in the culture supernatant were quantified by the plaque method. In the plaque method, HEL cells cultivated to flow into a 24-wells plate were adsorbed on a culture supernatant that was diluted for 1 hour at 37°C 1 hour and then superimposed with DMEM medium with 2% FCS and 0.4% agar for cultivation. After several days (6 to 12 days), the number of plaques displayed under the microscope was counted and the viral load measured.

Results: Comparison of the antiviral effect of GCV, Tricin, Anti-HCMV effects were investigated for several novel compounds found by docking simulation. Here the results that show the most significant effect are shown. The novel compound has been found to exhibit a stronger anti-HCMV effect compared to GCV and Tricin. Next EC-50 values were determined and compared.

Conclusion: The network pharmacological strategy integrated molecular docking to investigate the mechanism of action of AHI against COVID-19. It provides protein targets associated with COVID-19 that can be further tested as therapeutic targets of this safety ingredients.

Keywords: *Anti-Virus New CAM; Anti-Cytomegalovirus Agent; CDK9; Tricin; Corona Virus*

Abbreviations

CAM: Complementary and Alternative Medicine, in addition to Western medicine, there are many traditional medicine and/or health-promoting menus all over the world; COVID-19: COVID-19, A novel coronavirus pneumonia outbreak and clear statement by WHO; KCI:

Kumazasa Cam Ingredient; HCMV: Human Cytomegalo Virus; GCV: Ganciclovir; MERS: Middle East Respiratory Syndrome Coronavirus; SARS: Severe Acute Respiratory Syndrome Coronavirus

Aim and Scope

Novel coronavirus pneumonia (COVID-19) was first reported in Wuhan, China, in late December 2019 and has rapidly spread to more than hundreds of countries, including the United States, with thousands of infected people and hundreds of deaths within a month [1]. By June 15, 2020, more than 2 million cases had been identified in the United States, and a total of 50 thousands cases had been reported in Japan. It is of great importance to look for fast and effective therapeutic drugs for COVID-19. Drug recycling is a common strategy in the search for antiviral treatment. Network pharmacology is considered a promising approach to cost-effective drug development and has often been used to support the active ingredients of some Traditional Chinese medicine and its mechanisms of action. It has transformed the research approach "one goal, one drug" into a strategy "network goal, multi-components". Using the example of Kumazasa, the targeting method for natural products was used based on the PubChem database, the targeting method is fast and could provide a relatively accurate result without the support of high-performance computing. Based on the principle of structural biology, molecular docking can be used to perform virtual drug screenings via a computer-aided drug molecular biology design. Therefore, molecular docking is an effective way to find and identify drug targets by filtering the docking energy and space between molecules and targets. The integration of molecular docking and network pharmacology is helpful to accelerate the progress of target determination and experimental verification. In this study, a technology-based strategy integrated with molecular docking was used to investigate the mechanism by which AHI could potentially improve COVID-19 to provide scientific evidence for clinical drugs.

Coronaviruses are RNA viruses that are associated with the Religious Capsoviridae and coronaviridae. There are six types of coronaviruses that can infect humans, Human coronavirus OC43, Human Coronavirus 229E, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), Human coronavirus NL63 and Human Coronavirus HKU, SARS-CoV-2. The RNA genome is encapsulated in an envelope with polymorphic particles with a diameter of 60 - 140 nm. It is sensitive to UV radiation and heat and can be effectively inactivated by heat exposure at 56°C for 30 min, 75% ethanol, ether, chlorine-containing disinfectant and chloroform, but its structure, properties and biological properties remain can be fully clarified. From the perspective of nucleic acid sequence similarity, SARS-CoV-2 and SARS-CoV is more similar to MERS-CoV in CoV. The research showed that the SARS-CoV-2 nucleotide sequence had 79.5%, similar to SARS-CoV and the homology with bat-SL-CoVZC45 more than 85%. Like SARS-CoV, SARS-CoV-2 penetrates cells via the ACE2 receptor. This proposal summarizes demographic data and new features of SARS-CoV, MERS-CoV and SAR-CoV-2.

A selection of the health diet menu in conjunction with any composition is complex due to the lack of information about these cross-interactions between the public and healthcare menu. However, every individual is usually exposed to the risks of immunodeficiency status in their daily life due to internal and/or external factors. The factors that influence acquired immune activity are systemic metabolic disorders such as diabetes mellitus, malnutrition, extreme exhaustion, extensive stress, aging and medical side effects [2-10]. In this announcement we are pleased to establish the Committee for the Evaluation of Antiviral Agents from the Natural Product Kumazasa (*Sasa veitchii*). A target agent in our *in vitro* screening is the human cytomegalovirus (HCMV), known as human herpes virus 5 (HHV-5), which belongs to the family of herpes virus and is found in the majority of the world's population as a ubiquitous viral agent. Highly effective drugs such as ganciclovir (GCV), valacyclovir (VAL), Foscarnet (PFA) and cidofovir (CDV) are available for HCMV treatment. These compounds inhibit viral DNA synthesis by targeting HCMV DNA polymerase. However, the appearance of GCV-resistant viruses is a recurring problem in the treatment of immunocompromised patients. In addition, current antiviral drugs often cause unwanted lateral effects such as bone marrow suppression for GCV and nephrotoxicity for PFA and CDV [11-15]. Taking these problems into account, a novel type of anti-HCMV agent is immediately required. Cyclin-dependent kinase (CDK) 9 is known to be associated with viral replication and it is often used as a target protein for drug development. Recently, there is a report describing the interaction between CDK9/Cyclin T1 and viral pUL69 in HCMV. It has also been reported that CDK9/Cyclin T1 in mammalian cells initiates transcription expansion of genes by phos-

phorylation of the carboxyterminal domain (CTD) of RNA polymerase II in Ser2. These results suggest that Tricin is a promising candidate for new anti-HCMV drug development [16].

Materials and Methods

Target materials are plant materials derived from Kumazasa CAM Derivatives (KCI). The first prejudices for selection are based on the virus infectious diseases, such as small pox, coronavirus pneumonia, hepatitis type B, C and D, cancer, etc.

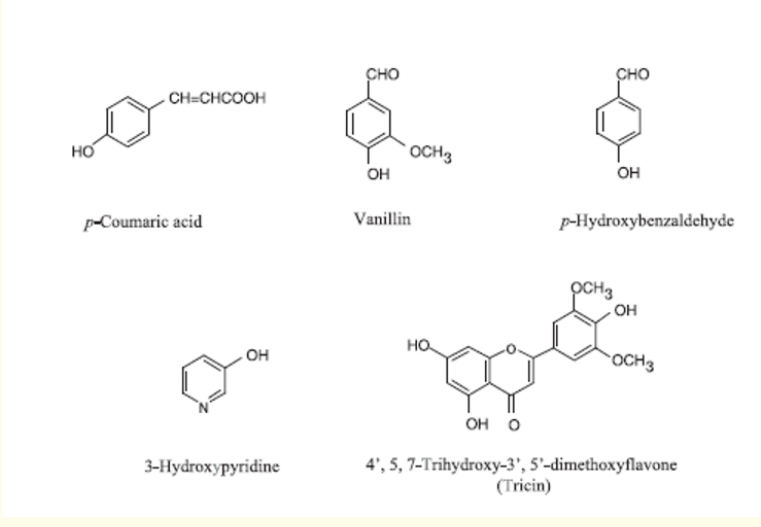

Figure 1: *Chemical compounds defined by KCI.*

Background and Invention

Objective: To date, we have found that tricin (4', 5,7-trihydroxy-3', 5'-dimethoxyflavon), one of the components contained in Sasa albo-marginata (Kumazasa in Japan), the expression of the human cytomegalovirus (HCMV), a strong antiviral effect against many DNA viruses and RNA viruses (varicella zoster virus, hepatitis B virus, coronavirus pneumonia virus, Zika virus, etc.), and its mechanism of action has been confirmed by a host factor that has not been explained so far (especially chemokine). Furthermore, since tricin is present in nature, no significant toxicity has been observed in *ex vivo* cell toxicity tests and toxicity tests in mouse *in vivo*.

Ganciclovir (GCV) currently used worldwide as an anti-HCMV drug has a strong specificity for HCMV infected cells and acts as a viral DNA synthesis inhibitor and exhibits strong anti-HCMV effects. However, as it is used over a long period of time, the pressure on medical expenditure and the appearance of viruses resistant to GCV is becoming a major social problem, and it is desirable to introduce new novel drugs with different mechanisms of action. Therefore, we looked for ingredients in herbal medicine and discovered tricin, which is more contained in Gramineae plants (we extract from Kumazasa), and as a result of the study it showed antiviral effects on host factor, chemokine, dependence, which I made clear. However, since the anti-HCMV effect of tricin is slightly weaker than GCV, it aimed to look for new compounds that show strong activity via GCV and Tricin.

Strategy: With Tricin as a key drug, we wanted to discover new compounds via Tricin and GCV.

Several candidate compounds with fluorine were found and synthesized from the docking simulation for cyclin-dependent kinase 9 (CDK-9) by imaging with the in silico, and the antiviral effect was compared and investigated.

Experimental method for anti-HCMV action

Human embryonic pulmonary fibroblast (HEL), obtained from human fetal lungs cultivated in 9.6 cm^2 shell until confluence with Dulbecco's Modified Eagle Medium (DMEM) medium was carried out, supplemented with 8% amount of fetal bovine serum (FCS). Towne strain of HCMV has been adsorbed and infected in a variety of infections (MOI) = 1 in HEL cells. After incubation at 37°C for 1 hour, concentrations of 0.1, 1μ, 10 μM of tricin or novel compounds were added and cultivated for 6 days, and infectious HCMV numbers in the culture supernatants were quantified by the plaque method. In the plaque method, HEL cells cultivated to flow into a 24-wells plate were adsorbed on a culture supernatant that was diluted for 1 hour at 37°C 1 hour and then superimposed with DMEM medium with 2% FCS and 0.4% agar for cultivation. After several days (6 to 12 days), the number of plaques displayed under the microscope was counted and the viral load measured.

Results

Comparison of the antiviral effect of GCV, tricin

Anti-HCMV effects were investigated for several novel compounds found by docking simulation. Here the results are shown with a novel compound Tricin that shows the most significant effect. The novel compound has been found to exhibit a stronger anti-HCMV effect compared to GCV and Tricin. Next, EC-50 values were determined.

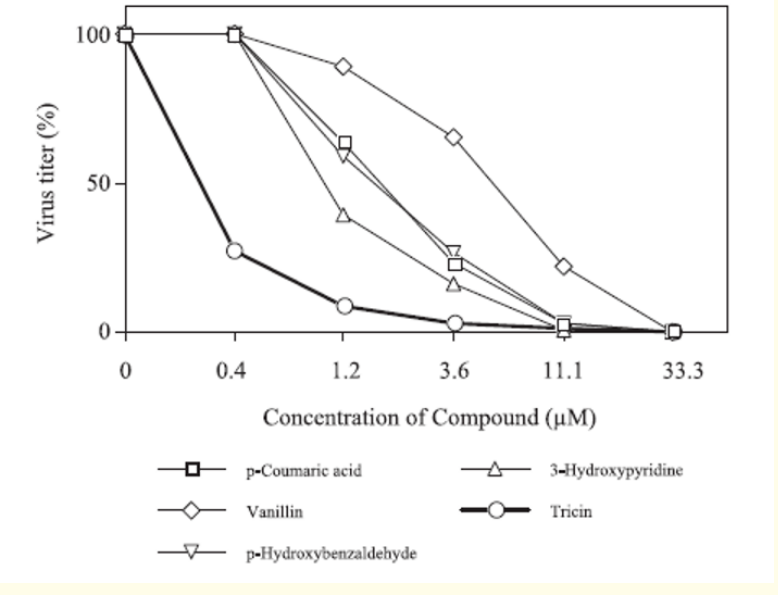

Figure 2: *Comparison of extracted compounds on anti-viral effect for HCMV replication.*

Figure 2 comparison of EC_{50} values of anti-HCMV activity. HEL cells were infected with HCMV at moi of 1 and then incubated with the specified concentrations GCV, tricin or 6F-tricin. Viral titters in the culture supersedes were determined by plaque assay on the 6th day after infection. The EC_{50} values of the anti-HCMV activity calculated by software of the NIH image J32.

The antiviral activity against HCMV of GCV, Tricin, a novel compound was compared and investigated for EC_{50}.

As a result, the EC_{50} values of GCV, tricin and novel compounds were 27.5, 54.3 and 0.13 nM, respectively. It has been revealed that the novel compound shows anti-HCMV effect of about 200 times GCV and about 400 times of tricin.

In addition, neither tricin nor novel compounds as a result of the study of cytotoxicity showed cytotoxicity and a direct injury effect on the virus to at least 10M.

Comparison of extracted compounds on anti-viral effect for HCMV replication

Effects on virus replication between the groups, time lag by KCI extracts/tricin. From the results from in figure 2, timing of treatment by Tricin was clear cut factor for regulation to virus replication.

Effects on virus replication between the groups, time lag by KCI extracts/tricin

Comparison of EC_{50} values of anti-HCMV activity. HEL cells were infected with HCMV at moI of 1 and then incubated with the specified concentrations GCV, tricin or 6F-tricin. Viral titters in the culture supersedes were determined by plaque assay on the 6th day after infection. The EC_{50} values of the anti-HCMV activity calculated by software of the NIH image J32.

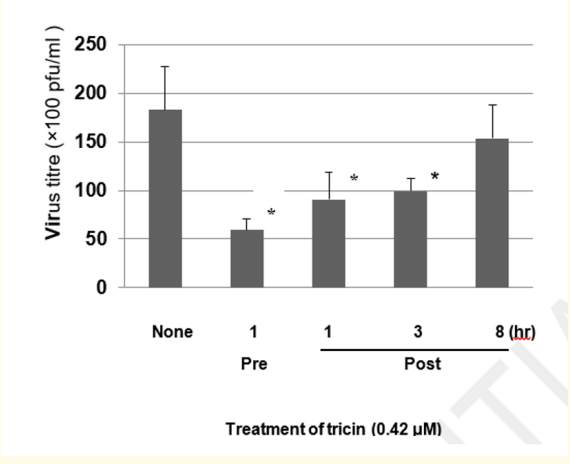

Figure 3: *Effects on virus replication the groups, time lag by KCI extracts/tricin.*

Effects on virus replication between the group, pre- and post tricin treatment

In order to search the mechanism of KCI on virus replication, at least regulation of replication and /or killing activity to the intact virus molecule.

The examination were designed to set on the various time course before treatment by KI to HCMV molecule.

Effects on gene replication between the group within major early gene and late gene expression

So as to test the Effects on gene replication between the group within major early gene and late gene expression, the experiments were designed to set up and shown in figure 5. As can see from figure 5A and 5B, the gene replication between the group within major early gene and late gene expression were indicated that early gene expression was regulated by the tricin molecule.

Mechanism of action

From the results of KCI, virus growth was inhibited to growth of HCMV through inhibiting replicate major early gene (IE). This mechanism of action was different from the growth inhibition by GCV.

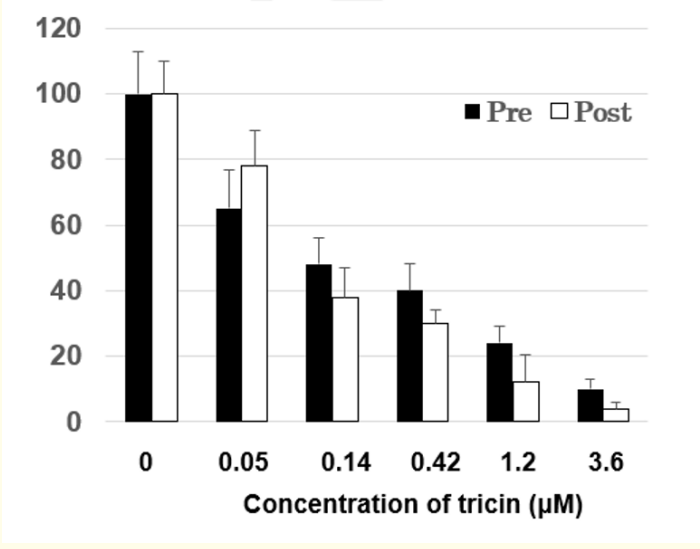

Figure 4: *Effects on virus replication between the group, pre- and post tricin treatment.*

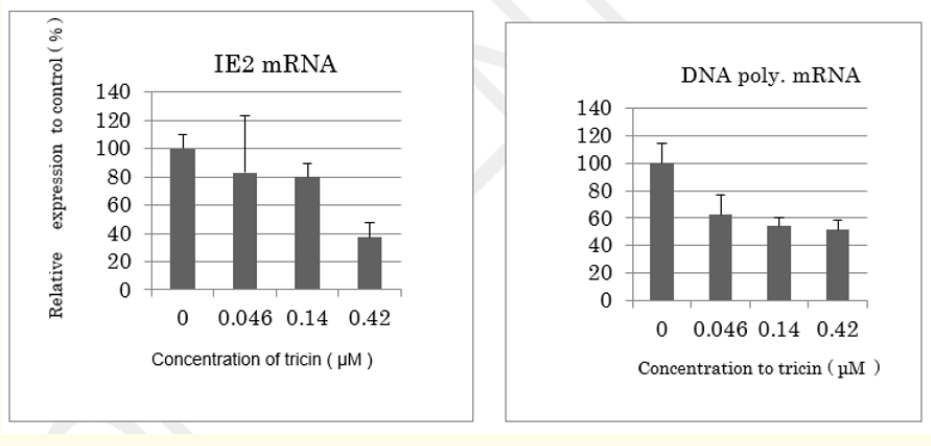

Figure 5: *Effects on gene replication between the group within major early gene and late gene expression.*

Direct toxicity of tricin upon nursing cells

Finally, the decoction had checked the toxicity against host cell, acute and multiple administrating one. This stand point was critical gate to get the royal road of the New Drug. From the results in figure 6, direct toxicity was not shown to virus molecule and the nursing cell human fibroblast.

The original material Kumazasa had been employed as natural package both for boiled rice especially portable rice cake/kind of Sushi Style featuring sour fish and/or traditional sweets in Japan. Safety test had been confirmed as food packages as well as family bare, including Panda, Family Ailuropoda.

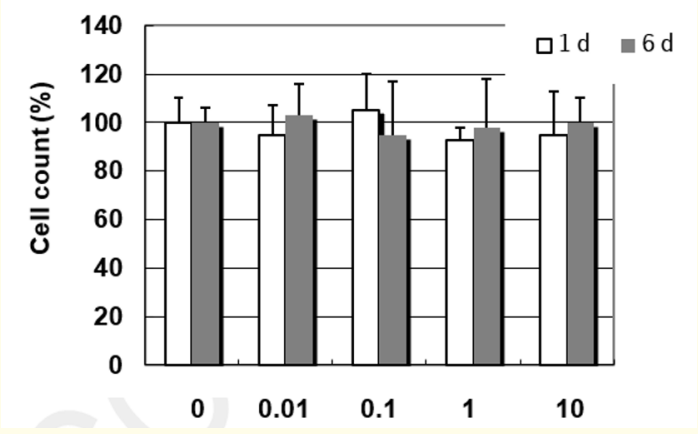

Figure 6: *Direct toxicity of tricin upon nursing cell.*

Discussion

Coronavirus pneumonia is a highly infectious acute respiratory disease caused by coronavirus. The World Health Organization estimates that about 1 billion people are infected with coronavirus pneumonia each year, coronavirus pneumonia resulting in 300,000 to 500,000 deaths. The virus is highly infectious and has a high mortality rate, making it one of the biggest infectious diseases that seriously threaten human health. The appearance of coronavirus pneumonia-resistant strains has been accelerated by the strong use of antiviral drugs such as oseltamivir. Therefore, it is particularly urgent to develop safe and efficient new anti-coronavirus pneumonia drugs.

In a world-wide trial, traditional Chinese medicine (TCM) has been used for thousands of years to treat pneumonia in coronavirus with good safety and remarkable efficacy. KCI comes from the book "Treatise on differentiation and treatment of epidemic febrile disease" and has been used for hundreds of years. At the same time, it is also one of the TCM prescriptions recommended treatment for coronavirus pneumonia in China and its anti-coronavirus pneumonia and anti-coronavirus pneumonia efficacy has been confirmed by clinical and experimental studies. In this study, network pharmacology was used to predict and investigate the targets of KCI against the coronavirus pneumonia virus and to investigate the mechanism of KCI in the treatment of coronavirus pneumonia, which provided a theoretical basis for subsequent *in vivo* and *in vitro* experimental studies of KCI regimen.

More and more studies have confirmed that TCM has a good effect on viral infectious diseases. A prospective, randomized, controlled study of 410 participants confirmed that KCI had a decoction of a shorter fever deposition time than oseltamivir in the treatment of coronavirus. The challenge experiment of the coronavirus. A virus also confirmed that KCI had a protective effect on mice. KCI could prolong the survival of infected mice and improve survival rates.

Currently, the Database of the Traditional Chinese Medicine Systems Pharmacology Database and Analysis is the most widely used database for screening active ingredient components and targets in network pharmacology research. The general method of network pharmacology research is to review the components for achieving oral bioavailability (OB) and drug similarity (DL) in the TCMSP database and then to verify their corresponding objectives. The SymMap platform integrates target information. Drug Bank and NCBI databases and is one of the largest target databases to date. However, only OB data from components is provided by this database, while DL data does not, and the corresponding targets of components cannot be retrieved directly. Therefore, we have skipped the step of screening active ingredients, and the goal with FDR (Benjamini-Hochberg Multiple Testing Correction) < 0.05 corresponds to any traditional Chinese medicine has been directly included.

This suggests that KCI may play an anti-coronavirus role coronavirus by regulating early gene expression [17-19].

Through the antiviral drug market, 5.7 billion people are a major market in the world. In addition, there will be a large market for drug delivery, even due to the bias for the frequency of the outbreak and the appearance of drug-resistant virus strains. The aim of this project is to supply a new antiviral agent KCI for the global market. The detailed amount of the production of antiviral agents includes: USD 568 million after domestic production; 351 million U.S. dollars from underwater production. The use and production of antiviral agents will continue to increase, an increase in the market of about five times the previous year [20-25]. The expanding KCI market also allows for veterinary use. The development of KCI for the current market is much more necessary to promote the spread of resistant strains to the former clinical antiviral agents.

Conclusion

From the above, it has been shown that tricin of a novel compound shows an anti-HCMV effect in a concentration of 1/200 to less than that of a known drug GCV widely used around the world, and no cytotoxicity has been observed. This suggested that the novel compound may be a promising new drug candidate. Safety test had been confirmed also.

Conflict of Interest

We don't explain any.

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