Possibility for New Drug Inhibiting Immediate Early Gene of HCMV

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Abstract: HCMV is a pathogen worth our attention because it's highly prevalent in the world, and threatens the health of immunocompromised people. However, the existing drugs have many disadvantages including toxicity and viral drug resistance. Therefore, we need to find a new effective drug for this pathogen. One such possibility comes from ESE drugs extracted from plant and is shown to be effective against herpesvirus.

Key words: immunocompromised, new drug, gene

1. Introduction

Human cytomegalovirus (HCMV) is a DNA virus that causes chronic infection in over 80% of people in the world. Normally, HCMV can be transmitted by sexual contact or body fluid such as saliva and urine; however, HCMV can also infect infants through breast milk, or adults through solid organ transplantation or blood transfusion. In immunocompetent people, HCMV primary infection usually causes mild flu-like symptoms and lead to an asymptomatic chronic infection. In rare circumstances, chronic HCMV infection in immunocompetent individuals can develop complications such as pneumonia, hepatitis and meningitis, however the worst diseases can occur in immunocompromised populations including people with HIV, transplant recipients and infants. In HIV-infected individuals, HCMV can causes severe symptoms by opportunistic infection. Transmission of HCMV to babies can also have severe consequences. HCMV primary-infected mothers have a 30% possibility of infecting their infants through placental transmission (Revello M G, et al., 2002; Revello M G, et al, 2002; Stagno S, et al., 1986) and congenital HCMV infection rate is estimated to be 0.6%~0.7% of births worldwide (Kim Phuong, et al., 2013). HCMV Infection during pregnancy can result in severe consequences, with approximately 12% of symptomatic babies die from the infection. In addition, 50% of the surviving infants can have permanent sequelae including developmental retardation, vision loss, sensorial deafness, or a combination of these effects (Ramsay M E, et al., 1991). HCMV also presents a big threat to transplant recipients. Approximately 75% of transplant patients are infected with HCMV during the first year after solid organ transplant. In this population, HCMV infection can also cause allograft rejection directly or indirectly, and make patients susceptible to opportunistic infections and malignancies. (Fishman JA, et al., 2007; Pereyra F, et al., 2004) Therefore, effective treatments for HCMV are critical to limit viral infection in these susceptible populations.

Although HCMV can infect tissues all over the body, salivary glands, which are responsible for saliva transmission, are key tissue targets for HCMV infection (Koichi, et al., 2007). HCMV disrupts the cytoskeleton in cells, causing infected cells to be uncommonly enlarged, which is a key attribute for pathology diagnosis whereas the symptoms of HCMV infection are similar with infectious mononucleosis or other glandular fevers. Among all host cell targets, fibroblasts are the most well studied in HCMV research. Fibroblasts are essential connective cells crucial for wound healing and play an important role in tumor cell mediation, as in the cases of tumor-associated host fibroblasts (TAF). TAF can induce extracellular matrix (ECM) remodeling to control tumor cell proliferation among epithelial cells (Silzle, et al., 2004). Infection of HCMV in fibroblast cells can interfere with their production of anti-tumor cytokines – ECM chemokines including Tenascin and Thrombospondin-1. Therefore, HCMV may eventually cause mucoepidermoid carcinoma. (Melnick M, et al., 2011)

HCMV can lead to a persistent infection in a host because it can evade the immune system using many different mechanisms. This means that a person once infected can be a potential carrier of this virus for the rest of their life. As a DNA virus, HCMV has a large genome of ~230kb. The HCMV genes can be classified into two types: unique long (UL) or unique short (US) genes. Various immune evasion mechanisms are controlled by multiple UL or US proteins. (Mocarski ES, et

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al., 2007) One evasion mechanism used by HCMV is to mimic the human Class I MHC using the UL18 viral protein. UL18 is expressed on the infected host cell's plasma membrane and has a better capacity than human Class I MHC for binding to the T cell and NK cell receptors to inhibit their activation (Chapman et al., 1999; Cosman et al., 1997). Even more complicated mechanisms for blocking the presentation of antigens are inside the cell, including US6 protein blocking peptide translocation by inhibiting ATP binding to TAP proteins. TAP proteins are structures responsible for pumping peptides into the ER to bind with MHC class 1. Additionally, US3 protein can inhibit functions of tapasin, an MHC class 1 antigen-processing molecule, and US2 and US11 can induce ER-associated degradation. (Ted H. Hansen., Marlene Bouvier. 2009)

Table.1 List of IE1 and IE2 functions

IE 1	IE 2
productive viral replication	productive viral replication
(under low MOI)	(main factor)
promote transcriptional activation with IE2	principal transcriptional activator of early gene
---	block host cell DNA replication
associates with mitotic host cell chromatin	binds to DNA
(protein-protein function)	
block extrinsic apoptosis	block extrinsic apoptosis
Antagonist of ND10-Related Intrinsic Defenses	---
(counteracting PML)	
antagonize the type I IFN response interfering with Jak-STAT signaling	Direct suppression of chemokine production
Summary on evasion strategies	
IE1--antagonization	IE2--inhibition
apoptosis	*apoptosis*
ND10-related transcription silencing	*inflammatory cytokine/chemokine induction*
type I IFN signaling	---

However, the earliest and most important immune evasion strategy for HCMV is the expression of proteins called immediate early protein (IE1 and IE2) which play an important role in the early infection of HCMV and several types of immune evasion. Both IE1 and IE2 are able to promote viral replication and transcription, but their exact functions are different (see Table 1). IE1 can interact with human signal transducer and activator of transcription (STAT) proteins to block host anti-viral gene expression. In addition, transcriptional regulation by IE1 and IE2 appears to interact with cellular transcription factors including histone-modifying enzymes to block their function. (Christina, et al., 2009; Fang, et al., 2016) IE1 can bind to host cell chromatin inside the nucleus to prevent limitations on viral gene transcription, whereas IE2 can bind to host cell DNA and directly inhibit antiviral responses by cooperating with cellular transcription factors. As a mechanism to block extrinsic apoptosis, IE1 can target the tumor suppressor protein PML; and IE2 binds to tumor suppressor p53 interfering with its transcriptional activator function (Christina, et al., 2009; Fang, et al., 2016).

Because HCMV can inhibit tumor suppressor proteins, it can protect tumor cells from apoptosis and create a microenvironment which promotes the development of tumor cells (Soroceanu and Cobbs., 2011). For example, HCMV-infected fibroblast cells persistently secrete factors including the glial cell derived neurotrophic factor (GDNF) which can promote tumor cell activities (Esseghir et al., 2007; Ng et al., 2009). In such circumstances, even immunocompetent people can suffer from latent HCMV infection.

2. Current drugs

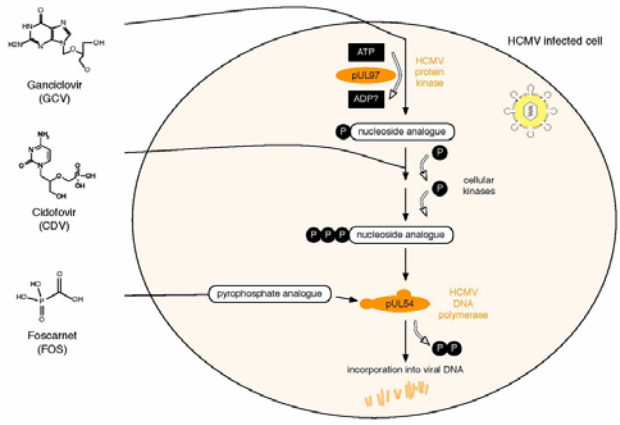

Fig.1 drug targets. (pic from Wikipedia). This picture briefly explained how three most prevalent anti-HCMV drugs function with viral replicating process

There are many drugs developed for HCMV including ganciclovir, acyclovir, foscarnet, and cidofovir. Ganciclovir and acyclovir are guanine analogues that can inhibit viral replication by targeting viral DNA polymerase. Cidofovir is a phosphorylated nucleotide analogue similar to ganciclovir and acyclovir that works by blocking DNA polymerase. Foscarnet is a pyrophosphate analogue that functions by blocking the pyrophosphate binding site of pUL54. Ganciclovir is used as a first-line treatment, but it needs phosphorylation by viral protein kinase, pUL97, to be activated, and as such the mutations reducing phosphorylation activity of pUL97 can lead to drug resistance. (Biron KK, et al., 1986). Due to toxicity, cidofovir and foscarnet are only given after ganciclovir resistance develops in an infected individual. Still, HCMV can mutate to develop resistance to these two drugs. As nucleic acid analogues, ganciclovir, acyclovir and cidofovir drugs may cause severe side effects by interfering with human DNA replication. Although these drugs can be used to treat infections in immunocompromised population, their usage still involves questions of toxicity, poor oral bioavailability, modest efficacy, and development of drug resistance (Andrei G et al., 2009). In addition, there is no drug approved for treating congenital infections and new trial drugs have failed to meet the expectations. (Christina, et al., 2009) Therefore, there is a need to develop new drugs to treat HCMV infection and evidence suggests IE1 and IE2 are ideal candidate drug targets.

Since the IE genes are so important for HCMV replication, there are many possibilities for finding drugs to inhibit IE gene expression. Current research has discovered potential drug activity of extract prepared from leaves of the Elaeocarpus sylvestris plant. Elaeocarpus sylvestris extract (ESE) was shown to inhibit IE gene expression and interfere with HCMV infection (Kim Phuong, et al., 2013). This research showed that ESE inhibited HCMV replication at a MOI of 1.0. ESE treatment reduced HCMV replication in HFF cells by 99.6% which is notably higher than the DMSO control. In this study, researchers found that ESE can interfere with the expression of viral lytic gene expression. Three types of lytic genes -IE, E, L – were significantly inhibited. More importantly, the ESE treatment did not seem to severely affect the viability of infected fibroblast cells. Treatment of fibroblasts with 10 μg/ml of ESE only slightly lowered the viability of these cells compared to the control group (Kim Phuong, et al., 2013)

Elaeocarpus sylvestris is a kind of tree which mainly grows in eastern Asian countries including China, Japan, and Korea. Elaeocarpus sylvestris is easily grown and can even be used as shade trees. Raw plant material would be abundant if needed to produce drugs, so the drug cost may be much cheaper than any exist drugs for HCMV. This species contains a potential active chemical called the gallotannin 1,2,3,4,6-penta-O-galloyl- beta-D-glucose, a compound that has previously suggested to be radioprotective and used in traditional Chinese medicine. Also, there is a research about effectiveness of this key compound on inhibiting replication of varicella-zoster virus (VZV) which is in the same family-herpesviruses- with HCMV (Bae S, et al., 2017). Therefore, ESE may be an effective and cheap drug with minimum adverse effects. We propose to test the efficacy of ESE and evaluate how this compound can be formulated into a drug.

3. Research

Although ESE has been discovered that it may help with HCMV therapy, there is still a long way for us to produce it as a drug.

3.1 Optimize effective dose of ESE

We need to test ESE's toxicity and effect further. In Kim's ESE research, the viability of fibroblasts treated with ESE was only slightly different from DMSO control, but the viability was still reduced by 32%. ESE would cause severe side effects if it were to kill 32% of the fibroblasts in our body. However, this outcome is only tested under one specific density of ESE. Therefore, we need to try different doses of ESE and observe its toxicity on fibroblasts versus the optimal dose for an effect on HCMV. Previous research tested the effect and toxicity of 10 μg/ml ESE on fibroblasts, and it can reduce almost all HCMV replication. We will be sure to test for lower doses to minimize toxicity. We will incubate the fibroblasts with the following ESE concentrations, 0.1, 0.5, 1, 5, 10, 20 μg/ml, and then use PCR to measure HCMV viral DNA. We will also use the CellTiter-Glo – an assay which uses fluorescently-labeled ATP added to cells - to determine the active metabolism of the fibroblasts. We will create a histogram of the results to find the best dose for treating HCMV and with least influence on viability of fibroblasts.

3.2 Test active compound of ESE

Previous research proposed that ESE extract can inhibit expression of IE genes, but how exactly the ESE can interact with HCMV infection is still unknown. In order to understand our new drug better, we can measure the levels of different viral proteins using Western Blot of HCMV-infected cells with and without ESE treatment to find out which proteins might be suppressed after ESE treatment. At the same time, we can use PCR to detect the level of IE gene RNA to determine if the drug inhibits the translation or transcription of certain IE genes/proteins. We will also test whether gallotannin 1,2,3,4,6-penta-O-galloyl-beta-D-glucose is the effective compound inhibiting HCMV replication by purifying the extract. We will titer the amount of gallotannin glycerin needed to inhibit HCMV infection in fibroblasts and add same volume of glycerin in control group. Then we will use western blot to evaluate changes in viral proteins. If the amount of viral protein is significantly lowered compare to the control, this

compound is proved to be effective on treating HCMV.

3.3 Design efficient extraction of ESE

We need to design an efficient method to extract effective compounds and condense them into an effective concentration that would be easy to take. In Kim's research, researchers used 70% ethanol to extract this drug. However, this extraction may not yield sufficient compounds because of the variation in plants and solubility of alcohol. Therefore, we need to find a method that can extract the maximum amounts of active compounds and retain their solubility and structure. At first, we can test what temperature the valid compound can bear, so we can determine whether it will survive the direct distilling from ethanol. If not, we can try extraction using other liquid with low boiling point such as benzene, then we can distill and condense the ESE drug. We can also tell which temperature is best for storage by doing this.

3.4 Test toxicity in uninfected mice

Third, we will begin to test the performance of ESE and gallotannin glycerinum in animals. Although the previous test on ESE shows that there are little side effects on fibroblasts, we can't determine what side effects ESE will cause on all kinds of cells and how the metabolism of the drug inside the body if we don't test it in a living subject. We will test the toxicity of these compounds in vivo by injecting 5 different concentrations of each compound into groups of uninfected 6 mice and then monitoring for symptoms of morbidity.

3.5 Test efficacy in animal models of HCMV

Because HCMV is a human only virus, there has no direct animal model for this virus. However, in the past several years, scientists designed humanized mice– immunocompromised mice engrafted with regenerating human tissue – to investigate this virus. We can use this method to prepare our mice models. We would then give mice different doses of our primary ESE drug to determine the lowest effective dose and the highest tolerable dose in vivo. The drug resistance of HCMV can be tested by persistent treatment in mice models. We can constantly give different effective dose of ESE to mice and measure density of viral particles in serum during a long period using PCR to discover how difficult and how long HCMV can develop resistance to this drug. If it shows that the drug can effectively control the HCMV replication without severe side effects, we can further test it in volunteers and patients with HCMV.

4. Summary

HCMV is a pathogen worth our attention because it's highly prevalent in the world, and threatens the health of immunocompromised people. However, the existing drugs have many disadvantages including toxicity and viral drug resistance. Therefore, we need to find a new effective drug for this pathogen. One such possibility comes from ESE drugs extracted from plant and is shown to be effective against herpesvirus. Consequently, I illustrated my idea and general design of my research in this proposal.

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