The Efficacy of Antiviral Components from Plant Products against Herpes Simplex Virus Type 1

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Abstract

HSV-1 is the leading transmittable cause of blindness in the United State and the developed countries of the world. HSV-1 establishes lifelong latency in the TG and is associated with high morbidity and mortality in humans worldwide. Currently, there is no method available to eliminate latent HSV-1 from an infected individual. Several FDA approved anti-HSV-1 drugs, such as Acyclovir, Valancyclovir, Famcyclovir and Pencyclovir are available for treatment. However, widespread use of these nucleoside analogues produces drug resistant HSV-1 mutants that cause more severe infections. Also these drugs have no ability to prevent latency/recurrences. To find a cure for this devastating disease, cost effective natural antivirals that prevent resistance development with reduced toxicity are of immediate need. A wide variety of plants are being studied as a source of natural products that can result in the development of novel drugs. Several compounds from these sources have shown high levels of anti-HSV-1 activity. However, development of antivirals from these herbal sources has not been completely explored. Recently, we have tested the efficacy of partially purified component(s) from regular and pearl garlic (PG) against HSV-1 in vitro. The compound(s) from pearl garlic extracts have shown strong antiviral efficacy against wild type HSV-1 and ACV resistant HSV-1 mutants as compared to regular garlic. About 70% of the ACV resistant TK- HSV-1 replication was inhibited by the isolated components from PG. Active constituents from this plant product may provide useful lead to the development of new and effective antiviral agent(s) against HSV-1. In the current review, the findings from this study along with some of the most promising compounds from herbal sources having anti-HSV-1 activity will be described.

ABBREVIATIONS

HSV-1: Herpes Simplex Virus Type 1; ACV: Acyclovir; TG: Trigeminal Ganglion; RG: Regular Garlic; PG: Pearl or Single Clove Garlic; Pfu: Plaque Forming Units; CPE: Cytopathic Effect; TLC: Thin Layer Chromatography

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is of global concern for its contagious nature, recurrence ability, mutability and silent epidemic potential. It is associated with high morbidity and mortality in humans. Infections caused by HSV-1 are sometimes fatal, especially in neonates and immune-compromised patients. In the United States (US) and the developed nations of the world, HSV-1 is the most common cause of corneal blindness [1]. Approximately 500,000 new cases of primary infections of HSV-1 are reported each year in the US alone. Sixty percent of the population in the US is infected by the age of 5 years and 90% by the age of 15 years. Following primary infection, the virus travels up nerves to the trigeminal ganglion (TG) where it establishes a latent infection lasting for life [2]. A hallmark of HSV-1 latency is the reactivation and return of the virus to the original peripheral site of infection, where recurrent disease may result. When HSV-1 is latent in the TG, the recurrent disease following reactivation can lead to scarring of the cornea and loss of sight [3]. An individual with latent HSV-1 can remain asymptomatic for a long period of time and currently there are no methods available to eliminate the latent HSV-1 from the TG.

Currently, several investigators are exploring ways to reduce the frequency, duration, and severity of outbreaks of HSV-1. At the molecular level, investigators have developed several strategies to control this disease process. These include 1) the use of a ribozyme that targets and cleaves the mRNA of essential genes in HSV-1, 2) engineered oligonucleotides that can target HSV-1 microRNAs and Latency associated transcripts LATs, 3)
a replication defective mutant of HSV-1 that can prevent active and latent infection and can reduce the frequency of reactivation in infected hosts, and 4) isolation of ICPO and LATs negative mutants to prevent latency/ reactivation [4-6]. However, all these strategies are still at the experimental stages and need many more improvements to make them effective. Clearly, much further experimental work with expensive animal models which mimic human diseases will be necessary to make these studies successful.

Several FDA approved antiviral drugs are available to treat HSV-1 infections. These include Acyclovir, Valacyclovir, Famcyclovir and Pencyclovir. However, widespread use of these nucleoside analogues has shown viral resistance and the development of HSV-1 mutants which cause much more severe infections especially in neonates and immune-compromised patients. Furthermore, these antivirals lack the ability to prevent establishment of latency/recurrent infections and they only kill the virus that is actively growing [7].

These drugs require initial activation by HSV-1 thymidine kinase enzyme (TK) and during treatments they generally produce ineffective results against HSV-1 strains that have decreased or no TK activity. The second HSV-1 enzyme that is mutated is DNA polymerase which incorporates triphosphates of these drugs into the viral DNA. However, these mutants are less common than the TK negative mutants. The development of TK negative and DNA polymerase negative HSV-1 mutants are common in immunosuppressed patients and is often life threatening. The frequency of occurrence of mutants is 3% in normal and 7-10% in immune-suppressed patients [8-12].

Various HSV-1 vaccine candidates such as, a live attenuated virus, a replicative defective virus and combination of subunit unit of viral proteins have been developed. As of 2014, several of these vaccine candidates are in different stages of development as they are being tested for safety and efficacy. A few of the vaccines are currently in clinical trials but none of them have demonstrated full effectiveness. The development of effective and safe vaccines against HSV-1 is not yet possible because of high costs, strain differences and the complexity of their production [13,14].

The development of viral resistance towards nucleoside analogue antiviral agents enhances the need for new effective compounds against HSV-1 infection. Thus, new antiviral agents exhibiting different mechanisms of action are urgently needed. One of the best strategies is to develop non-nucleoside antiviral agents from plant products that may be less likely to produce drug resistant mutants and still be effective against wild type HSV-1 strains as well as HSV-1 mutants produced by the nucleoside analogues.

A wide variety of ethno-medicinal plants are being studied as a source of natural products useful in the development of novel drugs. For the last four decades extensive research has led to the discovery of a few agents with anti-herpes virus activity and many of them exhibit complementary or overlapping mechanisms of action [15-21]. Several of the compounds either inhibit viral entry, viral genome synthesis, maturation or assembly. Many of these plant products in combination have been used as lead compounds because of their specific in vitro and in vivo activity with low toxicity and significant structural similarity [15]. Currently, only two reports have shown that a triterpene glycyrrhetinic acid from Liquorice root and an alkaloid from Stephanaceparanthra were promising candidates as anti-HSV agents against ACV resistant mutants of HSV-1 [22-23]. Garlic extract has also been shown to have anti-HSV-1 activity in vitro [24,25]. However, extremely high concentrations of the garlic extracts were required to produce antiviral effects and these high concentrations were also toxic to the cells.

Although no plant-derived drug is currently in clinical use to treat HSV-1 infections, thiazolylsulfonamide, phloroglucinol, and triterpeneglycyryrrhetin acid are promising herbal product candidates in preclinical trials, but they are not yet approved by the FDA [19]. Interestingly most of these compounds can block virus entry into host cells and/or specific cellular enzymes, alone or sometimes in combination with acyclovir, which is a very important aspect in the context of viral drug resistance. The compounds having alternative mechanisms of action, unlike synthetic antiviral agents, can be the potential candidates to tackle the threats posed by drug resistant herpes viruses. Further advantages of natural compounds are fewer side effects in comparison to synthetic medical drugs, and the production of synergistic effects for more positive treatment outcomes. In addition, natural products from herbal sources may even prevent the establishment of latency and reactivation of HSV-1.

In a previous study, two tetracyclic quinolizidine alkaloids were isolated and tested against herpes viruses in vitro. These compounds were potent inhibitors of beta herpes virus (HHV-6) and showed moderate activity against HSV-1 [26]. The efficacies of partially purified component(s) from regular and pearl garlic were determined against HSV-1 in vitro. As compared to regular garlic (RG), components(s) in the pearl garlic (PG) extracts showed strong antiviral potency against wild type HSV-1 and ACV resistant TK mutants of HSV-1 (unpublished observations, manuscript in preparation). To support these observations, selected portions of the data are presented below. Briefly, regular and pearl (single clove garlic) extracts in methanol were partially purified using silica gel column chromatography. Components from both extracts were eluted using methanol as a solvent. Thin layer chromatography (TLC) was used to detect components in the collected fractions. Components with similar Rf values were pooled, freeze dried and concentrated. The concentration determinations used for the efficacy studies were performed as described previously [26-29].

The cell toxicity of isolated compounds from RG and PG was determined using vero cells. Semi-confluent monolayers of Vero cells were exposed to isolated components at various concentrations (0.5 – 5 mg/ml/well), incubated for 1-3 days and counted. Data is presented in (Table 1). The compounds were non-toxic to cells up to 2mg/well.

The efficacies of isolated compounds were also determined using vero cells. Semi-confluent monolayers of Vero cells were infected with either HSV-1 (F17+) or ACV resistant mutants of HSV-1 at a multiplicity of 0.1 pfu/cell for 1 hour at 37°C. After the removal of residual virus, fresh media with isolated compounds at a concentration of 1mg/ml/well was added and incubated until the Cytopathic Effect (CPE) was observed. When the 60-
70% CPE was observed in the cultures without drugs (extracts), the viruses were isolated from infected cultures and viral titers were determined using a Plaque Reduction Assay. These data are shown in (Table 2).

As mentioned earlier, as compared to RG, the components from PG showed strong antiviral efficacy against wild type HSV-1 and ACV resistant TK- mutants of HSV-1. The components from PG were effective against wild type HSV-1 and TK- mutants of HSV-1, whereas the ACV was effective only against wild type HSV-1. These results also showed an impressive 70% inhibition of replication of ACV resistant TK HSV-1 mutants, (Table 2). It appears that the isolated components from PG extracts may be inhibiting viral replication because the extracts were added to the cells after the cells had been infected with the virus. The components from both RG and PG extracts have been further purified and analyzed. The data indicated distinct differences in the structures of the components of RG and PG. Currently, studies are in progress to characterize and determine their structures for further efficacy studies in vitro and in vivo. Future findings from this study may enhance our knowledge regarding the non-nucleoside compounds with antiviral activity and offer an opportunity to design a rational approach to therapeutic intervention of HSV-1 infection of the cornea which may reduce the frequency of blindness.

**DISCUSSION AND CONCLUSIONS**

Herpes Simplex Virus type 1 infections is the leading transmissible cause of blindness in USA. The limited efficacy of the current treatment of HSV-1 infection enhances the need for novel therapies. The development of drug resistant mutants of HSV-1 from these treatments has become a cause of concern and a challenge to clinicians.

It is important to develop non-nucleoside drugs from herbal sources that (a) resolve active infection more effectively, (b) do not produce drug resistant mutants (c) are effective against mutants produced by the currently available nucleoside drugs (d) have the ability to prevent the establishment of latency/ recurrent episodes and (e) are cost effective and safe. Based on this design, in this laboratory partially purified components from RG and PG were tested for the anti-HSV-1 activity in vitro. The components from PG have shown strong efficacy against wild type HSV-1 and ACV resistant HSV-1 mutants in vitro. The components were as effective as ACV for the wild type HSV-1 and inhibited 70% replication of the ACV resistant HSV-1 mutants. Further analyses of these extract indicated that the majority of PG components are polar and showed different structural characteristics. Further purification and structural analyses are being carried out to determine their structures and efficacies against HSV-1 in vitro.

When completed, this study and/or similar studies will enhance our knowledge regarding the non-nucleoside compounds with antiviral activity and will offer the opportunity to design a rational approach to therapeutic intervention of HSV-1 infections.

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**REFERENCES**


