USING MATRIX ASSAYS TO ANALYZE THE SYNERGISTIC EFFICACY OF ANTIVIRAL AGENTS AGAINST HSV-2

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Abstract  
Over 400 million people world-wide have genital HSV-2 infections. The current therapies against HSV-2 infections include Acyclovir (ACV), which is incompletely effective, and Foscarnet for ACV-resistant herpes virus strains, which causes nephrotoxicity. We hypothesize that the nucleotidyl transferase superfamily (NTS) inhibitors, ciclopirox olamine (#41), an FDA-approved drug for topical use, and piroctone olamine (#191) will synergize with acyclovir (ACV) in vitro to act as a safe and effective agent against HSV-2 infections. Previous studies have indicated that compound #41 and #191 were found to inhibit viral replication at an early stage against one or more targets. This data provided sufficient evidence to suggest that these compounds could be effective in suppressing HSV infection in combination with ACV. To test this hypothesis, I performed synergy assays that contained both drugs in Vero cells, and quantitatively analyzed titers using the CompuSyn application. This computational method produced a combination index (CI) value greater than 1 for each synergy assay, indicating antagonism. Additionally, FOS was shown to be ineffective against ACV-resistant strains of HSV. This experiment was performed once for each drug, and more rounds of synergy assays are necessary to make an accurate conclusion. The purpose of this research was to identify a new combination of drugs with antiviral properties that work synergistically to yield the lowest concentration of herpes virus. If one of these, or other NTS inhibitors is found to work synergistically with acyclovir, then it will be a critical step in developing effective antiviral drug therapy for herpes infection.

Introduction  
Combination therapies are frequently utilized in the treatment of viral infections. In this experiment, I combined the existing antiviral agent against HSV-2 infections, Acyclovir (ACV), with nucleoside analog compounds; ciclopirox olamine, #41, and piroctone, #191, in separate assays and analyzed the viral titer to test their combined effects. Additionally, I determined the EC50 of the compound, Foscarnet (FOS). These viral titers were quantified using the combination index theorem developed by Chou and Talalay. We found that combinations of the nucleoside analog compounds with ACV against HSV-2 were antagonistic, and not synergistic. Additionally, Foscarnet, is the current therapy for ACV-resistant HSV strains, however, our experiment showed that Foscarnet is not an effective agent against HSV infections. This analysis can be compared to similar biomedical research studies that analyzed the effectiveness of drug combinations used to treat HIV. At the Howard Hughes Medical Institute in the Department of Genetics at Harvard Medical School, the Xu lab examined nearly 500,000 drug pairs—identifying drugs that synergize
to inhibit HIV replication.\textsuperscript{1} They identified combinations of anti-inflammatory drugs that worked in pairs to synergize by targeting different steps of the HIV life cycle.

Existing drugs that treat HSV infections include Acyclovir (ACV) and Foscarnet (FOS). Acyclovir is an approved treatment for HSV infections—available as a topical dosage or an oral dosage. FOS is used for ACV resistant mutant strains, which act by inhibiting chain elongation of viral DNA polymerase; however, it is highly toxic. Ciclopirox (#41) has been used as a topical anti-myotic drug for onychomycosis (toe fungus infections), and studies show that it is safe to apply vaginally to treat yeast infections.\textsuperscript{1A} Piroctone (#191) is not approved for pharmaceutical use in the U.S.; however, it has also been shown to act as a safe anti-fungal agent. This research project is focused on analyzing the synergistic properties of drugs #41 and #191 because previous studies by the Morrison laboratory discovered that these drugs inhibit at least one event in herpes simplex virus replication that occurs at a very early, post entry stage of herpes virus replication, plus one or more events that occur during a later phase of viral replication.\textsuperscript{2} Additionally, #41 can suppress ACV-resistant mutant viruses suggesting the possibility of synergy with ACV.

\textbf{Methods}

A plaque assay to test for synergy was performed to assess the additive or synergistic activities of ciclopirox and piroctone with existing antivirals ACV or FOS in vitro. First, a plaque assay was necessary to determine the 50\% effective concentration value (EC\textsubscript{50}). Then, in a standard assay, I serially diluted the two compounds with PBS and added them to cells, either alone or in a combination ratio, including the virus. Next, I incubated the cells for 24 hours and collected all wells. Finally, I quantified the viral titer by plaque assays. I used the Chou-Talalay method for the matrix assays to compare the drugs’ combined effects. The Chou-Talalay method is a quantitative technique used to assess the additive and synergistic properties of the drug combinations. This method is derived from the median-effect equation which includes major biochemistry and biophysics equations.\textsuperscript{3} The calculated results from the titers will be plotted on a graph with the concentration of drug 1 on the x-axis and the concentration of drug 2 on the y-axis. A software program, CompuSyn (combsy.com/), will be used to interpret the data. The Compusyn software produced a concentration index (CI) value that will be used to determine synergy, additivity, or antagonism.

\textbf{Results}

The results of this experiment can be observed in \textit{Table A}. The combination index (CI) value for ACV and Compound #41 was 1.73, and the CI value for ACV and Compound #191 was 1.77. Since these values are greater than 1, they indicate antagonism. The EC\textsubscript{50} value was calculated for Foscarnet. This value is the inflection point on each graph. The EC\textsubscript{50} can be observed in \textit{Figure 1.0} and \textit{Figure 2.0}.

\textsuperscript{1} Tan, X. Hu, L et al. (2012). Systematic Identification of Synergistic Drug Pairs Targeting HIV. Nature Biotechnology, 30(11), 1125-1130.
\textsuperscript{1A} Subissi, A et al. (2010). Ciclopirox: recent nonclinical and clinical data relevant to its use as a topical antymycotic agent. Drugs. 70:2133-2152.
\textsuperscript{3} Chou, Ting-Chao. (2010). Drug Combination Studies and Their Synergy Quantification Using the Chou Talalay Method. The Journal of Cancer Research, 440-446.
Table A - AC Synergy Assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>CI Value</th>
<th>Synergy, Additivity, or Antagonism?</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACV + #41</td>
<td>1.73</td>
<td>Antagonism</td>
</tr>
<tr>
<td>ACV + #191</td>
<td>1.77</td>
<td>Antagonism</td>
</tr>
</tbody>
</table>

Discussion

This experiment was only performed once for each synergy assay. More rounds are necessary to conclude if synergy has occurred. Due to the ability for HSV to mutate to escape existing antiviral therapy, there is a significant need for anti-herpes virus drugs. This research can help develop new topical drug candidates to treat herpes infections in combination with existing drugs. HSV-2 infections are acquired through sexual contact. The prevalence of genital HSV-2 infections in the United States is about 22%, a 30% increase over the past decade. HSV-2 persistently infects 1 in 6 Americans and more than 400 million people worldwide. Incidences of infection are highest among black women, particularly of a lower socioeconomic status. Also, viral strains resistant to antiviral therapy are more common in immunocompromised individuals. This study is significant because it addresses the need for more effective antiviral drug treatment for HSV infections. Anti-herpes virus medications are needed now more than ever to effectively suppress primary and recurrent HSV-2 infections due to an increase in ACV-resistant virulent strains.
References


