# Acyclovir Resistance Due to Mutations in Viral Thymidine Kinase

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## ABSTRACT

Acyclovir is an antiviral medication commonly used in the treatment of Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). It is a guanosine nucleoside analog which functions to inhibit the viral DNA polymerase and stop viral growth. Acyclovir is an important drug because HSV-1 and HSV-2 are responsible for many viral infections in the US patient population, and these infections can lead to serious cor

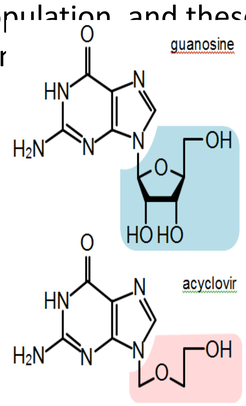


Figure 1. Comparison of acyclovir and guanosine

Acyclovir is an inactive prodrug that needs to be converted to acyclovir-triphosphate in the cells, which requires the virus-encoded thymidine kinase (vTK). A mutation in vTK can cause HSV infections that are resistant to acyclovir treatment.

## INTRODUCTION

A neonate presented to the hospital with skin blisters on the forehead and upper lip. Through lumbar puncture testing, HSV-1 DNA was positively identified in cerebral spinal fluid and the patient was diagnosed with neonatal herpes encephalitis (NHE).

Acyclovir was the chosen drug for treatment. After a few days, the symptoms improved. However, the viral DNA remained in the CSF four weeks after the initiation of acyclovir therapy due to the development of HSV-1 resistance to the drug (1).

In the United States, the number of herpes infections testing positive for HSV-1 and HSV-2 is estimated to be 50% and 20%, respectively (2).

The most common infection sites for HSV-1 are the mucous membranes of the mouth, nose, or eyes. HSV-2 is most commonly associated with infections in the anogenital region, but both HSV-1 and HSV-2 can cause serious

## MOLECULAR STORY

HSV infection begins with fusion of viral encoded glycoproteins to host cell membranes. Infecting capsids travel through the nuclear pores and transport the viral genome into the host cell nucleus. Viral DNA is then replicated and more infectious capsids are produced. The latency associated transcript is expressed to cause recurrent infections of HSV. Acyclovir fights HSV infections by blocking replication (2).

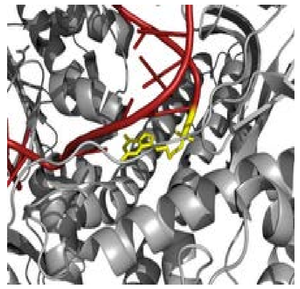

Figure 2. Acyclovir (yellow) shown incorporated into a DNA helix (red) complexed with DNA polymerase (gray). This view illustrates how acyclovir is substituted for guanine into viral DNA at the 3' end but does not allow for further nucleotide addition, therefore halting DNA polymerase and viral DNA replication.

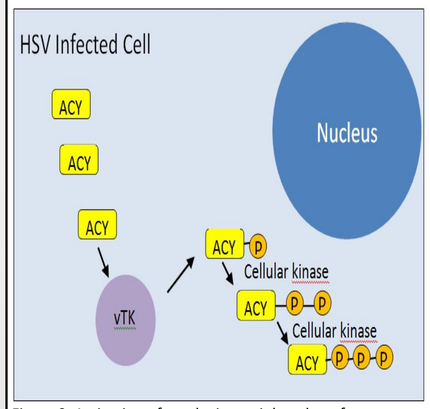


Figure 3. Activation of acyclovir to triphosphate form

- Acyclovir is a prodrug that requires phosphorylation (3)
- vTK binding is essential to activity of acyclovir because vTK phosphorylation is the first step in activation of acyclovir
- Requires two additional phosphorylations by cellular kinases
- Triphosphate form is incorporated into viral DNA and blocks DNA polymerase because it lacks 3'-OH

- Hydrogen bonds responsible for most interactions in vTK active site
- Most pertinent interactions at Gln125, Arg176, Tyr172, and Tyr101
- Tyr172 interacts via Van der Waals forces
- vTK phosphorylates acyclovir at 5'-OH

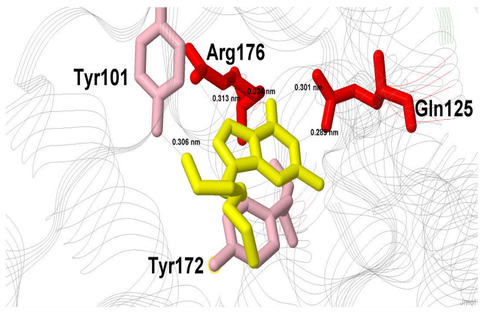


Figure 4. Close up view of acyclovir in the binding pocket of vTK. This view emphasizes the active site amino acids (red) that can mutate: the underlying cause of drug (acyclovir) resistant HSV-1 strains.

- 95% of acyclovir resistance cases associated with vTK mutations (4)
- In patient case, mutation resulted in Gln125His leading to impaired acyclovir binding (1)
- Arg176Trp also shown to be associated with resistance (4)

## UNADDRESSED CLINICAL ISSUES

The Gln125 to histidine mutation did not induce cross-resistance to ganciclovir, penciclovir, or brivudin suggesting that these drugs may be effective against this mutation. If mutations were to occur in the future that made non resistant medications ineffective, continuing antiviral medication development would be essential. It may be a beneficial approach to find why the above medications did not show cross resistance.

Herpes simplex is not a curable disease state because it has a latent phase which cannot currently be targeted by drug therapy. During latency, the latency-associated transcript (LAT) is expressed which causes recurrent cases of HSV-1 (5). It may be beneficial to develop a drug target for the LAT gene.

Continued d at do not require phos neficial for patients at ri nfections. Antivirals tha es instead of viral kinases could potentially work for resistant infections.

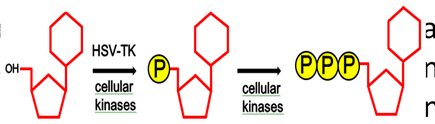


Figure 5. Acyclovir is activated by viral thymidine kinase

## SUMMARY

A substitution of thymidine at nucleotide G375 in the vTK gene resulted in an amino acid change of Gln125 to histidine (1). This mutation is critical because Gln125 is an amino acid that is essential for the binding of acyclovir to the vTK active site. If acyclovir cannot bind to the active site, then the drug no longer can maintain its function and continue be therapeutic. Altho clovir has been proven effective, it st y n sms for resistance.

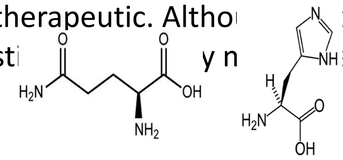

Figure 6. Comparison of glutamine (left) to histidine (right)

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