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Review

Resistance of human cytomegalovirus to ganciclovir/valganciclovir: A comprehensive review of putative resistance pathways ☆

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ABSTRACT

Human cytomegalovirus (HCMV) is a pathogen that can be life-threatening in immunocompromised individuals. Valganciclovir and its parent drug ganciclovir are currently the principle drugs used for the treatment or prevention of HCMV disease. The development of HCMV resistance to ganciclovir/valganciclovir has been documented in treated patients and is associated with the emergence of amino acid substitutions in the viral proteins pUL97, pUL54 or both. Generally, single amino acid substitutions associated with clinical resistance that alone do not confer decreased ganciclovir susceptibility in cell culture have been disregarded as causative or clinically significant. This review focuses on the analysis and mechanisms of antiviral drug resistance to HCMV. We also conducted a review of publicly available clinical and nonclinical data to construct a comprehensive list of pUL97 and pUL54 amino acid substitutions that are associated with a poor clinical response to the first line therapies ganciclovir and valganciclovir, or associated with reduced HCMV ganciclovir susceptibility in cell culture. Over 40 putative ganciclovir/valganciclovir resistance-associated substitutions were identified in this analysis. These include the commonly reported substitutions M460I/V and C592G in pUL97. There were additional substitutions that are not widely considered as ganciclovir/valganciclovir resistance-associated substitutions, including V466M in pUL97 and E315D in pUL54. Some of these ganciclovir/valganciclovir resistance-associated substitutions may confer cross-resistance to other HCMV therapies, such as cidofovir and foscarnet. Based on this review, we propose that there are more potential HCMV ganciclovir/valganciclovir resistance pathways than generally appreciated. The resulting comprehensive list of putative ganciclovir/valganciclovir resistance-associated substitutions provides a foundation for future investigations to characterize the role of specific substitutions or combinations of substitutions, which will enhance our understanding of HCMV mechanisms of ganciclovir/valganciclovir resistance and also provide insight regarding the potential for cross-resistance to other HCMV therapies.

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☆ The views expressed in this article are those of the authors and do not necessarily reflect the official position of the FDA.

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1. Introduction

As antiviral therapy has become widely used in various viral diseases, including disease associated with human cytomegalovirus (HCMV) infection, it has also become crucial to understand the mechanisms and clinical consequences of antiviral drug resistance, including the relevant virus, host and drug-related factors. Knowledge of the mechanisms of antiviral drug resistance has allowed for development of genotypic and phenotypic techniques for the timely diagnosis of resistance. Consequences of drug resistance for any viral infection may range from toxicity inherent in the use of antivirals designated as second-line in treatment guidelines to severe disease and even death from progressive viral infection when no effective alternative treatments are available. While the mechanisms and clinical impact of antiviral drug resistance have been well described for human immunodeficiency virus (HIV-1), hepatitis C virus (HCV), hepatitis B virus (HBV) and influenza virus, they have been less well characterized for HCMV even though drug-resistant HCMV is also a major clinical concern.

Cytomegaloviruses are members of the *Herpesviridae* family, and are capable of causing a variety of acute, latent and recurrent infections in humans and animals. HCMV, also designated as the human herpesvirus 5, is the prototype of the betaherpesvirus group (Roizman et al., 1981). The HCMV genome is about 230 kb and encodes more than 250 open reading frames. HCMV infection can be primary or secondary. Primary infection occurs in a HCMV seronegative susceptible host, whereas secondary infection represents reactivation of a latent infection or reinfection of a seropositive host.

As a general rule for all viral infections and antiviral drugs, antiviral drug pressure can select for the emergence of pre-existing drug resistant variants by conferring a survival advantage to those subpopulations that are relatively less susceptible to the antiviral agent. Alternatively, spontaneous changes in the viral genome generated during viral replication in the presence of drug selection can lead to the emergence of a drug resistant viral population. In either case, secondary consequences of resistance-associated nucleotide or amino acid substitutions can include alterations in viral pathogenicity, transmissibility, and genetic stability. Development of antiviral resistance can often increase in frequency and significance as antiviral agents are utilized more widely. Great strides have been made in the methods to characterize the mechanisms and development of antiviral drug resistance using biochemical assays or cell culture models, but there are still many limitations to these approaches. While these assays have become more sensitive, they often do not identify certain indirect mechanisms that may contribute towards clinically relevant drug resistance, such as compensatory viral fitness amino acid substitutions (Bloom et al., 2010), viral substitutions that only confer resistance when present in combination with other substitutions (Kobayashi et al., 2011; Sun et al., 2012), or viral- or host-specific (e.g., ribavirin, (Ibarra et al., 2011)) changes that impact drug metabolism. As an example of a potentially indirect mechanism of drug resistance for HCMV, the pUL54 K805Q substitution improved the fitness of a virus harboring the pUL54 T821I substitution associated with resistance to foscarnet (Martin et al., 2010a), although the clinical relevance of this particular observation remains unclear.

While many of the concepts and approaches to studying antiviral drug resistance generally apply to all viruses, HCMV brings along several unique challenges. In this article we review the current state of the field regarding the treatment of HCMV disease, specifically focusing on the study, mechanisms and clinical consequences of antiviral drug resistance. We also conducted a review of publicly available non-clinical and clinical data to construct a comprehensive list of amino acid substitutions that are associated with resistance to the most commonly used treatments for HCMV infection, ganciclovir and valganciclovir, and we discuss the potential for cross-resistance to other HCMV therapies. Finally, we highlight some of the major knowledge gaps in HCMV drug resistance to help stimulate further studies, which may ultimately help guide the development of new therapies and rational strategies that address the challenges of HCMV drug resistance.

2. HCMV infection and disease

The prevalence of HCMV infection is estimated to vary from about 45% in developed countries to near 100% in developing countries. Primary infection usually occurs during the first decades of life and humans are believed to be the only host of the virus. Transmission sources of HCMV include saliva, urine, semen, cervical and vaginal secretions, milk, stool, blood, cells, tissues, and organ transplants. Primary infection in immunocompetent adults is mainly asymptomatic or it may be associated with a self-limited mononucleosis-like syndrome and leads to a life-long latent infection. However, in patients with immature or compromised immune systems (i.e., congenitally infected newborns, patients with AIDS and transplant patients) HCMV infections are often symptomatic, causing a range of symptoms such as colitis and pneumonitis, and are associated with increased morbidity and mortality.

2.1. Congenital HCMV infection

In the United States, it is estimated that approximately 44,000 infants are born each year with congenital HCMV infection (Stagno and Britt, 2006). Approximately 10% of infected newborns are symptomatic at birth. Mortality in symptomatic infants is about 12% and approximately 90% of survivors experience significant morbidity from the infection, including mental retardation, sensorineural hearing loss, microcephaly, seizures, or other medical problems. No drugs are FDA-approved for antiviral treatment of congenital HCMV infection. However, a report by Kimberlin and his colleagues (Kimberlin et al., 2003) suggests that 6 weeks intravenous ganciclovir may have a role in treatment of neonates with symptomatic HCMV infection.