Aciclovir resistance phenotype of clinical isolates proven by targeted mutagenesis of recombinant herpes simplex and varicella zoster viruses

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Abstract

The members of the human pathogenic α-herpesviruses herpes simplex virus (HSV) type 1 and 2 as well as varicella zoster virus (VZV) establish persistent infections of sensory ganglia and cause recurrent vesicular rashes. Especially immunocompromised patients suffer often from severe infections and need prolonged antiviral therapy at high dosage resulting in the emergence of resistance that is associated with sequence alterations of the affected viral enzymes. The nucleoside analog aciclovir (ACV) is the first line drug in such cases. Extended therapy often results in multiple amino-acid exchanges in the ACV-converting viral thymidine kinase (TK) of HSV. This multiplicity of polymorphisms and the difficulty of isolating clinical VZV hamper the precise assignment of the responsible nucleotide mutation to its resistance phenotype.

Here, the impact on ACV resistance of clinically relevant amino-acid mutations in the viral TK was assessed by the generation of virus recombinants that harbor one single TK mutation in an otherwise wild-type background. For this purpose, the genomes of HSV-1, HSV-2, and VZV which were cloned as bacterial artificial chromosome (BAC) were used for traceless sequence modifications by en passant mutagenesis in bacteria. First, a fluorescence reporter gene was inserted into a suitable genome position to monitor viral replication and to facilitate subsequent susceptibility testing. Based on the fluorescence-labeled and the basic BACs, the native TK-encoding genes were deleted and substituted with genes that harbored one single desired mutation. After virus reconstitution in permissive eukaryotic cells, the fluorescent virus strains exhibited replication capacities similar to their non-fluorescent counterparts and to the recombinant parental virus with wild-type configuration. Intact TK expression was demonstrated by detection of TK-specific transcripts and proteins. Furthermore, the correlation between the relative fluorescence intensity and the viral load was shown. Using the fluorescent virus strains, the antiviral action of ACV was examined by measuring the relative fluorescence. The impact of single amino-acid exchanges on ACV resistance was determined by calculating the 50% effective concentrations and by quantifying the viral loads in presence or absence of ACV.

Thus, each selected amino-acid mutation with so far ambiguous significance was precisely attributed to a natural or to a resistance-associated polymorphism for each virus recombinant generated. This forms the basis for improved molecular resistance diagnostics and for an improved guidance of antiviral therapy.