



***IN VITRO* SELECTION OF BRINCIDOFIVIR-RESISTANT AND CIDOFOVIR-RESISTANT HUMAN ADENOVIRUS**

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Background: Brincidofovir (BCV, CMX001) is an orally bioavailable lipid acyclic nucleoside phosphonate which is converted intracellularly to the active antiviral cidofovir diphosphate (CDV-PP). BCV shares the broad-spectrum antiviral activity of CDV against all five families of dsDNA viruses which cause disease in humans, including adenoviruses (AdV). The 50 to 500-fold improved *in vitro* activity of BCV vs CDV is likely due to efficient transport of circulating BCV across the cell membrane, resulting in higher intracellular concentrations of CDV-PP. Mutations in the AdV DNA polymerase gene have been reported to impart resistance to cidofovir (CDV). Since the active antiviral is qualitatively the same for BCV and CDV, but quantitatively different, sequence changes in BCV-resistant and CDV-resistant viruses selected under identical conditions were compared.

Methods: A laboratory strain of human adenovirus species C (AdV 5) was passaged in A549 cells in the presence of increasing concentrations of BCV or CDV for more than five months (15 passages). Every five passages the AdV polymerase gene sequence and EC₅₀ of passaged virus for BCV and CDV were determined using standard methods.

Results: Virus following the final passage with BCV or CDV exhibited a 4 to 8-fold increase in EC₅₀ versus wild-type virus. Genotyping identified three amino acid changes in the AdV polymerase sequence: T87I and V303I in BCV-passaged virus and T1150I in CDV-passaged virus. These viruses were not overtly growth impaired compared to the parent AdV strain based on DNA levels after identical growth periods. One of the changes (V303I) was reported previously for CDV resistance in AdV5 by Kinchington. The other two mutations have not been reported and suggest involvement of multiple regions of the AdV pol in determining resistance to these agents.

Conclusions: Different mutation patterns were detected in resistant AdV isolates selected by BCV and CDV, although one of two mutations selected by BCV had been previously reported for CDV. The reasons for the observed difference in mutations selected could include higher levels of CDV-PP in BCV-treated cells, stochastic selection or other unidentified causes. This is the first report of selecting BCV-resistant AdV in cell culture.