INTRODUCTION

Brincidofovir 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 BCV across the cell membrane resulting in higher intracellular concentrations of CDV-PP than wild type (WT) virus. Mutations in the AdV DNA polymerase gene have been reported to impart resistance to CDV (1, 2). Since the active antiviral agent is qualitatively the same for BCV and CDV, sequence changes in BCV-resistant and CDV-resistant isolates under similar conditions were compared.

METHODS

Passaging AdV 5 in BCV and CDV

175 flask were seeded with 2E+06 A549 cells. After overnight growth at 37°C, the cells were infected with AdV 5 at an MOI of 0.01. At the end of the infection period, cells were mixed with medium and 20% of fresh medium containing the appropriate concentration of CDV or BCV was added and incubation continued at 37°C. Flasks were harvested when protein and nucleic acid were detected in the media.

To determine if there was an increase in EC50 over the wild-type (WT) strain. The DNA polymerase region was sequenced in vitro selected virus and compared to the WT reference virus.

Phenotyping assay

The AdV phenotyping assay was carried out in A549 cells with a qPCR readout to quantitate the amount of AdV DNA. The assay was used to report the EC50 and fold-change (FC) of the test specimen compared to the WT reference virus.

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