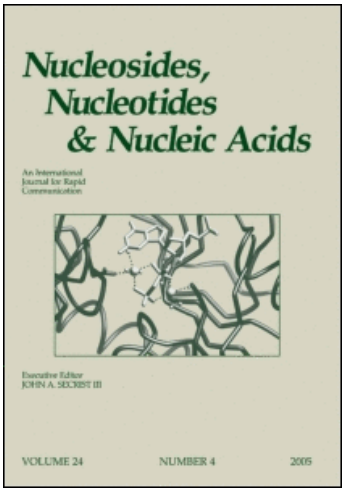

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Resistance of Human Cytomegalovirus to D- and L-Ribosyl Benzimidazoles as a Tool to Identify Potential Targets for Antiviral Drugs

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Resistance of Human Cytomegalovirus to D- and L-Ribosyl Benzimidazoles as a Tool to Identify Potential Targets for Antiviral Drugs

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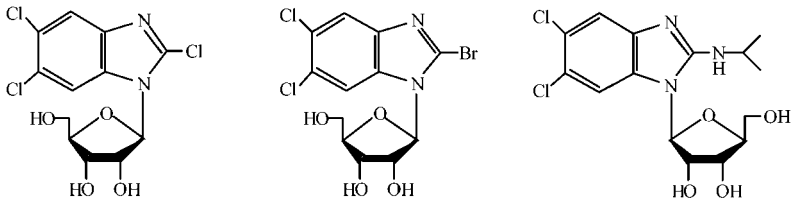
We and our collaborators have previously described the activity of D- and L-ribosyl benzimidazoles (TCRB or BDCRB and 1263W94, respectively) against human cytomegalovirus. Resistance to TCRB was mapped to genes UL56 and UL89[1,2] and resistance to 1263W94 was mapped to UL97.[3]



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In order to further investigate mode of action of D- and L- ribosyl benzimidazoles we have isolated an HCMV strain termed G2, which is resistant to both TCRB and 1263W94. This was accomplished by passaging HCMV strain C4 (with the UL56 and UL89 mutations) in increasing concentrations of 1263W94. A long-term growth study of HCMV strain G2 revealed that it was not growth deficient when compared to wild type HCMV. Genotypic characterization of this strain confirmed the presence of the expected mutations in UL56 and UL89 genes, but no mutations in gene UL97 were detected. Other genes involved in HCMV DNA synthesis and packaging (UL37 exon 1, UL44, 57, 98, 105 and UL51, 52, 77, 93, 104; respectively) were sequenced and no mutations that could explain resistance to 1263W94 were found. However, a previously undetected L21F mutation in gene UL104 was identified in both G2 and C4 virus isolates. An HCMV strain with only this mutation (UL104 L21F rec.) was constructed utilizing a recently cloned HCMV genome as a bacterial artificial chromosome (HCMV AD169-BAC).[4] This virus was as sensitive to BDCRB as the wild type HCMV (Fig. 1), indicating that this UL104 mutation did not play a role in BDCRB resistance.

In order to narrow the region of the G2 HCMV genome with the mutation responsible for 1263W94 resistance, a cosmid library of this strain was constructed. This cosmid library was then used together with deletion mutants made using AD169-BAC to construct recombinant viruses with portions of the wild type HCMV genome replaced with G2 cosmid DNA. This approach had narrowed the mutation to open reading frames UL26-UL31. Sequencing revealed only a single mutation, in gene UL27. Studies are underway to determine the currently unknown function of the protein encoded by this gene. We conclude that the protein encoded by gene

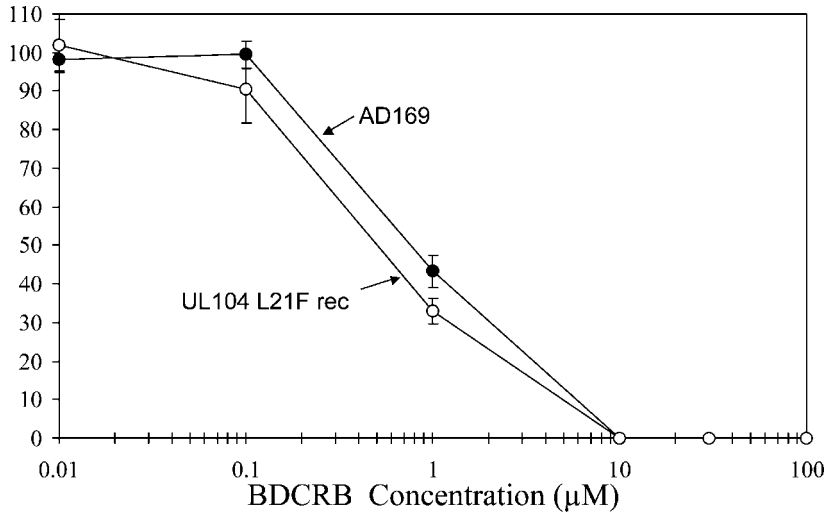

Figure 1. Activity of BDCRB against wild-type virus (AD169) and HCMV with L21F mutation in gene UL104 (UL104L21F rec.). Results of triplicate determinations from a plaque reduction assay are presented.

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UL104 is not a target for these antiviral drugs, but that the gene product of UL27 most likely is.

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