

Antiviral Activity of Amphotericin B Methyl Ester (AME): Isolation of AME-Resistant Mutants

Abdul A. Waheed¹, Sherimay Ablan¹, Akira Ono¹, Carl P. Schaffner², and Eric O. Freed¹

¹Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD 21702; ²Department of Microbiology and Biochemistry, The Waksman Institute of Microbiology, Rutgers-The State University of New Jersey, New Brunswick, NJ 08903

Previous studies from our lab demonstrated that membrane cholesterol plays an important role in HIV-1 assembly, release, and infectivity. Amphotericin B methyl ester (AME), a water-soluble, relatively non-toxic derivative of the polyene antibiotic amphotericin B (AB), is a cholesterol-binding compound. Antiviral activity of AB and its derivatives against a range of enveloped viruses has been reported; however, little is known about the mechanism of inhibition. To investigate the effect of AME on the HIV-1 replication cycle and to evaluate the potential for AME as an antiretroviral, we determined the effect of AME on HIV-1 replication in various T-cell lines and in primary cells. We observed that virus replication was potently inhibited. We also found that AME profoundly impaired HIV-1 infectivity in single-cycle assays in CD4+ HeLa-derived TZM cells. To investigate the effect of AME on the late stages of the virus replication cycle, we performed virus release assays in HeLa and Jurkat cells. We observed a ~5-fold decrease in virus particle production, with no significant effect on Gag binding to the plasma membrane, Gag association with lipid rafts, or Gag multimerization. To study further the antiviral properties of AME, we selected for AME-resistant virus in Jurkat T-cells. The mutations responsible for AME resistance, P203L and S205L, mapped to an endocytosis motif in the cytoplasmic tail of gp41. Virus replication and single-cycle infectivity assays confirmed that the P203L and S205L substitutions conferred AME resistance. Interestingly, truncation of the gp41 cytoplasmic tail of either HIV-1 or SIV also conferred resistance. This study sheds light on the target and mechanism of action of AME and provides support for the concept that cholesterol-binding compounds should be pursued as antiretrovirals.