tified by real time RT-PCR (Taq-Man) at 2 days with indicated dose (0.25 nM, 25 nM, 50 nM, and 250 nM) or with DMSO as control. Taq-Man PCR analysis showed a dose-dependent inhibition of HCV RNA replication after treatment with 17-AAG. The 17-AAG-mediated HCV replication inhibition is due to the directly interaction of Hsp90 and NS3 by immunoprecipitation. These results have exciting implications for the HCV life cycle and novel antiviral strategies.

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Development of Anti-Infective Topical Microbicides 2. Quantifying Inhibition of Virus Transmission in Microbicidal Setting

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Over 25 million people have died since the first case of AIDS was identified in 1981, and the number of people living with HIV worldwide continues to expand—from 35 million in 2001 to an estimated 40 million in 2005. In the absence of a fully effective HIV vaccine, topical microbicides represent an important potential strategy for preventing the transmission of HIV through sexual intercourse, the predominant mode of HIV transmission worldwide. Although a comprehensive understanding of HIV transmission has not yet emerged, it is currently thought that virus transmission occurs rapidly and is likely the result of infection of monocyte-derived cells in the vaginal mucosa by CCR5-tropic viruses. Transmission of virus in the microbicide setting will require agents which prevent virus entry, fusion, reverse transcription, or other pre-integrative events, or agents which directly inactivate HIV or modulate the target cells to render them uninfectable. In vitro assays which are typically utilized to evaluate the ability of a microbicide to prevent virus transmission utilize adherent cells (MAGI or GHOST cells) or cells more relevant to the development of anti-HIV therapeutics (PBMCs) and quantify virus production following transmission at short time intervals following infection. We have modified a virus sterilization assay to evaluate virus transmission over the course of 30 days of culture in the presence and absence of microbicidal compounds. These assays effectively demonstrate that the transmission inhibition capacity of microbicides is dramatically over-estimated in the short-term assays. Data obtained in this microbicidal transmission assay define the concentration of the microbicide required to completely prevent the transmission of virus to target cells and is likely the minimal concentration of the microbicide that will need to be provided in a final formulated product. Further the assay can be used to define the effects of viral multiplicity of infection and the effects of rare populations of resistant virus strains on transmission events. The results of our studies with a variety of potential microbicides being developed in our laboratories will be presented.

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Development of Anti-Infective Topical Microbicides 1. Effects of Seminal and Vaginal Fluids and Other Additives on Viral Infection and Drug Efficacy

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Over 25 million people have died since the first case of AIDS was identified in 1981, and the number of people living with HIV worldwide continues to expand from 35 million in 2001 to an estimated 40 million in 2005. Almost 5 million people worldwide became newly infected with HIV and an estimated 3.8 million human deaths were attributed to AIDS in 2005. In the absence of a fully effective HIV vaccine, topical microbicides represent an important potential strategy for preventing the transmission of HIV through sexual intercourse, the predominant mode of HIV transmission worldwide. The number of women with HIV infection and AIDS has been increasing steadily worldwide, accounting for 46% of all adults living with HIV worldwide, and for 57% in sub-Saharan Africa. Thus the dynamics of the epidemic demand the development of safe, effective and acceptable female-controlled chemical and physical barrier methods, including topical microbicides, to reduce HIV transmission. Although a complete understanding of virus infection of cells in the vaginal vault has yet to emerge in the microbicide field, it is accepted that infection likely occurs inefficiently in the first moments following the introduction of the viral inoculum in the vagina. In order to further explore the biology of infection, we have evaluated the effects of seminal plasma, vaginal fluids, other mucopolysaccharide additives and vaginal pH on both the infectivity of HIV and the efficacy of microbicides being developed for clinical use. Each of these additives or environmental changes might be expected to play roles alone and in combination with one another on the efficiency of virus transmission. The effects of these environmental conditions can be modeled and quantified in vitro. Our results would indicate that authentic seminal plasma has a major impact on the ability of HIV to transmit to target cells in assays which mimic the vaginal environment. Vaginal fluids have less effect on transmission but both biological fluids can affect the activity of potential microbicides in both negative and positive ways. We will present the results of studies demonstrating the impact of biological fluids on virus transmission and microbicide efficacy.

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