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Optimization of the Reverse Transcriptase Assay for the Detection of Viral Burden in Mice Infected with Rauscher Murine Leukemia Virus E.L. White, L. Westbrook, M.G. Hollingshead, and W.M. Shannon. Southern Research Institute, Birmingham, AL USA.

Rauscher murine leukemia virus induces an erythroleukemia in susceptible strains of mice resulting in splenomegaly and viremia. This animal model has been used in the evaluation of the *in vivo* efficacy of potential anti-HIV agents. The *in vivo* antiviral activity of therapeutic agents has usually been determined by measuring a reduction in the spleen weights of compound-treated mice when compared to vehicle-treated, infected controls, or by quantitating viremia with the UV-XC plaque assay. The UV-XC assay, however, is time-consuming and labor-intensive. The virions of Rauscher murine leukemia virus, like other retroviruses, contains the enzyme reverse transcriptase. Quantitating the level of this enzyme in infected mouse sera should provide a more rapid measure of viremia in the animal. Initial attempts, based on published methods, to quantitate the amount of reverse transcriptase in mouse sera were unsuccessful. Subsequently, we examined the effects of a number of reagents, including detergent, KCl, EGTA, dGMP, GMP, spermine, as well as protease and RNase inhibitors, on the reverse transcriptase assay. The optimized assay method was found to be effective in evaluating the antiviral activity of AZT in the Rauscher murine leukemia virus *in vivo* model. The assay is also amenable to being automated if a large number of assays are required. **This work was supported, in part, by NCI Contract No. NO1-CM-87274 and U.S. Army Medical Research Institute of Infectious Diseases Contract No. DAMD17-88-H-8003.**

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Antiretroviral Efficacy and Pharmacokinetics of Ester Prodrugs of 9-(2-Phosphonylmethoxy-ethyl)adenine (PMEA)

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The potent and selective antiretroviral activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) have been demonstrated in various *in vitro* and *in vivo* systems. Several ester prodrugs of PMEA that contain a lipophilic group linked at the phosphonate moiety have now been synthesized and evaluated for activity against human immunodeficiency virus (HIV) type 1 and type 2 in human T4 lymphoblast MT4 cells. The 2-trifluoroethyl, 2-trichloroethyl and 2-tribromoethyl esters of PMEA were found to have marked anti-HIV activity, the EC_{50} (concentration required to inhibit HIV-induced cytopathicity by 50%) ranging from 6 to 9 μg/ml, that is at a concentration >10-fold lower than the CC_{50} (or concentration that causes reduction of cell viability by 50%). Also, the compounds showed marked inhibitory activity against MSV-induced transformation of murine fibroblast C3H/3T3 cell cultures. When evaluated in MSV-infected newborn mice, the 2-trifluoroethyl and 2-trichloroethyl esters of PMEA were highly efficient in delaying MSV-induced tumor initiation and associated death of the mice. For instance, when administered as a single dose of 20 mg/kg on day 0 (the day of virus inoculation), the mean day of MSV-induced tumor initiation was 9.9, 8.3 and 7.9 days for PMEA, 2-trifluoroethyl-PMEA and 2-trichloroethyl-PMEA, respectively, as compared to 4.5 days for untreated control mice. Plasma concentrations of the ester compounds and their parental metabolite PMEA were determined by HPLC with fluorescence detection. The PMEA esters were easily degraded to PMEA, as demonstrated by a rapid appearance of PMEA in the plasma after intravenous bolus injection of the test compounds. The esters were also rapidly eliminated from the plasma, the elimination half-life being ~ 4 min for 2-trichloroethyl-PMEA and 2-trifluoroethyl-PMEA, as compared to ~ 12 min for the parent PMEA, following intravenous administration. Also, oral bioavailability of the PMEA esters was poor, the concentrations of PMEA in the plasma being < 1 μg/ml after oral gavage of a dose of 100 mg/kg. Whether the ester prodrugs can be taken up as such by the cells, or only after cleavage to PMEA, remains to be further investigated.