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HIV-TAT DRUG INTERACTION STUDIES USING 1G5 CELLS

Cell Culture and Transfection:

1G5 cells were maintained in RPMI 1640 supplemented with 10% HyClone fetal calf serum. Transfections were done in complete medium in a BTX electroporator at 120 V, 3000 µF, in 125 µl at 2.5 x 10^7 cells/ml in a 0.2 ml cuvette. Infections with retroviral vectors were done by supernatant exposure in complete medium containing 4.0 µg/ml polybrene. HIV infections using the NL4-3 laboratory strain were performed in complete medium. The infected cells were then treated with anti-viral compounds.

Luciferase Analysis:

Five days after infection with HIV or a Tat retroviral vector (E. Aguilar-Cordova, unpublished data) and exposure to antiviral compounds, the cells were harvested, centrifuged at 1800 rpm for two minutes, washed once with PBS, and resuspended in 50 µl of luciferase lysis buffer [25 mM Tris-phosphate (pH 7.8), 2 mM 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid, 10% glycerol, 1% Triton X-100]. The mixture was incubated at room temperature for 15-30 minutes and microfuged for 30 seconds. 10 µl of the lysis supernatant was added to 50 µl of luciferase assay reagent (Promega) and immediately read in a Packard scintillation counter set for single photon counting. All experiments were done in triplicate.