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A NEED FOR A MULTI-PARAMETER VIROLOGICAL TESTING IN SCREENING POTENTIAL AIDS DRUGS IN VITRO. D.J.Volsky, N. Hamblet, B. Volsky, M.G. Pellegrino, G. Li; Molecular Virology Laboratory, St Luke's/Roosevelt Hosp. Center and Columbia Univ. Coll. of Physicians and Surgeons, New York, NY.

Many compounds are active *in vitro* against human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS. However, only one drug - 3'-azido-3' deoxythymidine (AZT) - has been thus far approved for AIDS treatment. The antiviral activity of many compounds is inconsistent *in vitro*; other potential drugs are effective *in vitro* but not *in vivo*. Drug screening tests are usually limited to the evaluation of a single parameter of the HIV-1 infection, such as cell killing, cell fusion or virus replication,and thus do not duplicate the complexity of the HIV-1 life cycle. We have evaluated a drug screening protocol involving the analysis of multiple parameters of the HIV-1 life cycle during primary and chronic infections *in vitro*. T-cells or monocytes were infected with HIV-1, challenged with a drug, and evaluated for: a) viral genome transcription, by measuring the levels of intracellular HIV-1 RNA by liquid RNA hybridization; b) virus production, by measuring the intracellular and extracellular levels of HIV-1 p24 antigen, by p24 ELISA; c) proportion of HIV-1 antigen-positive cells by IF; and d) cell viability. Chronically infected T-cells (ACH-2) or monocytes (U1.1) were exposed to the drug or to supernatants from cultures of PBLs pre-treated with a drug, and tested as above, with or without HIV-1 inducers. T cells transfected with infectious HIV-1 DNA were evaluated for specific drug effects on the post-entry stage of the HIV-1 cycle. The drugs studied were: the RT inhibitors AZT, ddC and Suramin; immunomodulators THF and Ampligen; anti-cancer drug Lentinan; the anti-bacterial drug Rifabutin; and novel drugs interferring with the regulatory functions of HIV-1. The antiviral activities of each of the RT inhibitors were similar when measured by the 3 virus detection methods 2 days p.i.: EC_{50} of 0.01 μM for AZT, 0.1 μM for ddC and 12.5 μg/ml for Suramin. When measured 7 days p.i., the EC_{50} for AZT was 10 μM by RNA measurement, 0.1μM by IF, and 0.01 μM by p24 assay. THF had no effect on HIV-1 replication when added directly, but some supernatants from THF-treated PBLs transiently induced HIV-1 secretion, but not the rate of HIV-1 transcription. Rifabutin showed a dose-dependent inhibition of HIV-1 expression in acutely but not chronically infected cells. Lentinan had no effect on HIV-1 expression in any system tested. In conclusion: screening of prospective anti-HIV drugs requires testing multiple parameters of the HIV-1 infection cycle. Non-antiviral AIDS drugs should be evaluated for their potential to indirectly induce HIV-1.

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Synthesis and Enzymology of New Dideoxynucleosides with Anti-HIV Potential. V. Nair, G. S. Buenger, D. F. Purdy and T. B. Sells, Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242, U. S. A.

2',3'-Dideoxyadenosine (ddA) has been found to have potent antiviral activity against the human immunodeficiency virus (HIV-1). This activity is apparently due to the ability of ddA, as its triphosphate, to behave as a viral DNA chain terminator thereby inhibiting the action of HIV reverse transcriptase, the viral DNA polymerase. In a program in our laboratory directed at the chemistry and biology of dideoxynucleosides, we have synthesized new analogues of ddA bearing strategic modifications in both the carbohydrate and base moieties. The rationale for the design of these compounds in terms of structure, enzymology and potential for antiviral activity will be discussed. Details of representative syntheses, including key photochemical and metal-mediated transformations and their scope, will be illustrated and explained. Confirmation of the structures of the target compounds by spectroscopic methods including UV, FTIR, FAB HRMS and multinuclear high-field NMR will be briefly discussed. Relative rate data for glycosidic bond cleavage will be reported and analyzed. Substrate and inhibitor (competitive or non-competitive) activities of the target molecules towards mammalian adenosine deaminase will be presented and explained. The in vitro anti-HIV activity of selected target compounds together with structure-activity correlations will be discussed.