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Biological Measurement of Drug Sensitivity of HIV-1 in Peripheral Blood Mononuclear Cells of a Patient by the Plaque Hybridization Assay. S. Kato,[1] Y. Hiraishi,[1] T. Sugita,[1] M. Asakawa,[1] H. Hanabusa,[2] and T. Takano.[1] Department of Microbiology, Keio University School of Medicine,[1] and Pediatrics, Ogikubo Hospital,[2] Tokyo, Japan.

Detection of drug-resistant HIV emerging in patients receiving antiretroviral therapy is crucial in clinical setting. The drug resistance of HIV-1 is usually evaluated by changes in the amino acid sequence of the reverse transcriptase (RT). However, such genetic information does not give us the exact degree of drug sensitivity of viruses. We developed a biological assay for drug resistance of HIV-1, which is based on the plaque hybridization technique. This method allows us to quantitate viruses that can replicated in primary peripheral blood mononuclear cells at given concentrations of any soluble antiviral agents and to isolate each resistant virus clone. By using this assay, we monitored the drug resistance of HIV-1 in a patient who received antiretroviral therapy. After 8 months of AZT administration, the amino acid substitution in RT which is a mrker of AZT resistance was detected but HIV-1 in this patient was still susceptible to 0.01 μM AZT. After 13 months, the HIV-1 became resistant to 0.05 μM AZT. Suggested from these results, we changed AZT to DDI as the treatment of the patient. After 3 months of DDI treatment, about 20% of infectious HIV-1 became resistant to 10 μM DDI but no amino acid substitution specific for DDI resistance was detected in RT. These results indicated that there were significant discrepancies between the genotype and the phenotype with respect to drug resistance of HIV-1. The biological analysis of drug-resistant HIV-1 by our method should be useful for the proper design and the efficient monitoring of therapeutic interventions.

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EVALUATION OF LONG-TERM TREATMENT WITH GLYCYRRHIZIN AND OF COMBINATION THERAPY WITH GLYCYRRHIZIN AND AZT OR DDI ON HIV-1 CARRIERS

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The efficacy of oral monotherapy with glycyrrhizin (GL) (150-225mg/day) for 5 to 10 years and combination therapy with GL (for 5 to 10 years) and AZT (2-3 years) or ddI (1 to 2 years) on hemophiliac HIV-1 carriers was evaluated based on the measurement of the levels of viral RNA in plasma samples by branched DNA (bDNA) nucleic acid hybridization assay, and of the ability of the interferon productions by unfractionated blood cells in cultures in the presence of inducers (Sendai virus for IFN-α, PHA for IFN-γ). Results: 1) As regards the 6 asymptomatic HIV-1 carrier patients (AC pts) who started GL monotherapy at an AC stage (>500/μl of $CD4^+$ cell counts), the HIV-1 vRNA were undetectable in 4 AC pts, and were very low levels (11-12 keq/ml) in 2 AC pts. $CD4^+$ counts of all 6 AC pts were over 400/μl for 11 years, and the levels of IFN-α production were 80 to 175% of the mean value of control (uninfected hemophiliacs). 2) As regards other 7 pts (3 AC, 4 ARC) on the combination therapy, vRNA were detected in 2 ARC pts. $CD4^+$ counts of ARC pts have maintained over 200/μl, and those of AC pts, over 400/μl. The levels of IFN-α production of ARC pts were 20-30% of the control levels. AZT also induced the AZT resistant mutants in 2 ARC pts. Summary: Monotherapy with GL started early in AC stage have been effective to maintain their AC state for more than 11 years without induction of drug resistance and any side effects.

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CGP 61755, a Potential Combination Partner for Saquinavir: A Synergistic HIV-1 Protease Inhibitor with High Antiviral Potency

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The *in vitro* profiling of CGP 61755 reveals synergism with other HIV inhibitors, defined resistance-associated mutations in the protease gene and a favorable cross resistance pattern. Infectious molecular clones of clinically relevant protease mutants were generated by PCR-based *in vitro* mutagenesis and used for profiling by infection of human lymphoid cell lines, of fresh human PBL and macrophages.

CGP 61755, a hydroxyethylene derivative, produced by 10 step chemical synthesis, is specific for HIV-1 protease with an IC_{50} of 1 nM. The ED_{90} in acute infections of lines or primary cells is 30-100 nM. Even chronic HIV-1 infections of primary human macrophages are fully suppressed with 1 μM. Electron microscopy and protein analysis provide the mechanistic proof of protease inhibition. The ED_{90} value is 3fold higher in the presence of α_1-Acid- Glycoprotein (1mg/mL). A combination of CGP 61755 with RT- and protease inhibitors reveals synergy with AZT and Saquinavir (Invirase), and additive to synergistic effects are found with ddC, ddI, and Indinavir. Oral application of 120 mg/kg to mice provided plasma concentrations of 1-20 μM for up to 8 hrs. Formulated in a microcrystalline suspension CGP 61755 reaches up to 80 % bioavailability in female dogs, with a C_{max} of 8-19 μM and a t_{max} of 2 hrs. No adverse effects are seen in an 18 day rising dose (30-300 mg/kg) toxicology study in dogs. A broad antiviral *in vitro* profile is confirmed with drug resistant HIV-1 protease variants. CGP 61755 remains fully effective against the Invirase-resistant 48V/90M protease double mutant. Our evaluation therefore supports CGP 61755 as a suitable and potent partner for drug combination in antiviral therapy. Chemical manufacturing properties, as well as the target specificity of CGP 61755 in conjunction with favorable protein binding and a satisfying pharmacokinetic profile provide a desirable inhibitor profile. Moreover, a good potential for drug combination, based on the cross resistance profile, justifies a further evaluation of CGP 61755 in humans.

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Combination Effects of Vesnarinone with Zidovudine or Stavudine (D4T) on HIV Replication in Peripheral Blood Mononuclear Cells. M. Nokta[1], K. Loesch, W.C. Buhles[2], M. Yoshitake[2], and R.B. Pollard, [1]University of Texas Medical Branch, Galveston, TX and [2]Otsuka America Pharmaceuticals, Inc., Palo Alto, CA, USA.

Vesnarinone (VSN), a quinalone derivative used for treatment of patients with congestive heart failure, was recently reported to possess immunomodulatory properties, was shown to inhibit HIV replication, and to enhance the rate of AZT phosphorylation in vitro. In this communication the effect of VSN on the Zidovudine (AZT) and d4T induced inhibition of HIV replication was examined. Phytohaemagglutinin activated peripheral blood mononuclear cells were infected with HIV strain MN for 1 hour and then were incubated in the presence or absence of either VSN (10-250 μM), AZT, (0.001-5 μM) or d4T (0.15-3 μM) alone, or in a combination mixture of VSN/AZT or VSN/d4T. At 1 and 2 weeks postinfection, HIV replication was determined by measuring p24 Ag in culture supernatants by EIA. VSN, at doses of 12.5 μM to 250 μM, in combination with 0.001 to 1 μM AZT had a synergistic effect on the inhibition of HIV replication. Moreover VSN at doses between 62.5 to 250 μM enhanced the inhibitory effects of 0.15 to 1.5 μM d4T on HIV replication. VSN at the doses tested were not cytotoxic. VSN, is currently being tested in combination with Didanosine and Zalcitabine. These data suggest that VSN at doses achievable in humans may be clinically useful in combination therapy for patients with HIV disease.