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Ribonucleotide Reductase Inhibitors, Didox and Trimidox, Demonstrate Antiretroviral Activity Alone or in Combination with DDI in a Murine Acquired Immunodeficiency (MAIDS) Model. H. Elford[1], B. Van't Riet[1], C. Mayhew[2], O. Oakley[2], N. Hughes[2], J. Piper[2], V. Gallicchio[2]. [1]Molecules for Health, Inc., Richmond, VA; [2]University of Kentucky, Lexington, KY.

A new treatment approach to HIV infection is based on the thesis that the quantity of deoxynucleotides can be made deficient, thereby impairing the synthesis of proviral DNA by inhibiting cellular ribonucleotide reductase (RR). This premise has gained recognition by clinical trials in which RR inhibitor hydroxyurea exhibit anti-HIV activity in combination with didanosine (DDI). In this study, we tested Didox and Trimidox, potent RR inhibitors, alone or in combination with DDI in the murine immunodeficiency model of AIDS (MAIDS). Infected mice were treated (M-F) with Didox or Trimidox alone or in combination with DDI. Didox or Trimidox treatment alone produced marked increase in survival and a strong suppression of viremia (splenomegaly) as well as reduced lymphadenopathy and hypergammaglobulinemia. Ten weeks or less of combination treatment decrease the expression of viral gag 12 envelope glycoprotein to undetectable levels. The antiviral effect was enhanced with DDI; however, DDI alone had only marginal activity. All the untreated MAIDS infected animals and those treated with DDI alone had died by week 32 while a significant number of Didox, Trimidox treated singly and in combination with DDI lived beyond one year. These results further support the therapeutic potential of these drugs.

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Pharmacokinetic properties and toxicological studies of GPI2A, a gene expression modulator and a potent antiviral agent against HIV-1 virus.
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New drugs that modulate gene expression and virus replication are required as tools to study HIV-1 proliferation and as potential therapeutic compounds. This report describes the preclinical development of GPI2A, a drug candidate against the HIV\AIDS disease. GPI2A is a 20-mer antisense oligodeoxynucleotide partially thioated at seven base positions, and is designed to target a highly conserved region of the HIV-1 genome. Antisense activity was demonstrated in the B4.14 cell line that constitutively expresses the HIV proteins. Inhibition of multiple HIV (strains IIIB, NL43 and JRFL) replication was shown in infected-PBMC. Reductions in the RT-activity and the viral p24 protein levels in HIV-1 infected cells demonstrated the antiviral activity. Pharmacokinetic properties were characterized with labeled GPI2A in CD-1 mice. GPI2A distributes to most tissues including the brain, with high concentrations in the thymus after a single i.v. or s.c. of 30 mg/kg body weight injections. GPI2A persisted in the blood and plasma for up to 9 hours and persisted in tissues for 7 days postinjection. Single doses of 250 mg/kg body weight were tolerated in mice. A daily subcutaneous injections of 30 mg/kg body over 14 days has been well tolerated in CD-1 mice without any noticbale toxicity.

Conclusion: GPI2A is a potent inhibitor of HIV-1 gene expression and replication with a good pharmacokinetic profile and it is non toxic. Laboratory studies with GPI2A have shown encouraging results, and clinical trials are scheduled to begin shortly to evaluated the therapeutic benefits. This work was supported by a research grant from the Manitoba Health Research Council to J.A.W.

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Pentafuside (T-20), A Novel Inhibitor of HIV-1 Fusion: Pharmacokinetics in Rodents, Monkeys and Man. T. Venetta, B. DiMassimo, M.R. Johnson, D.M. Lambert, M.A. Recny, S. Hopkins and M. Saag* Trimeris, Inc., P. O. Box 13963, Research Triangle Park, NC 27709 *AIDS Outpatient Clinic, University of Alabama at Birmingham, Birmingham, Alabama 35294

The T-20 LAB (Labeled Avidin Biotin) ELISA was developed for use in detecting and quantifying T-20 in biological fluids. The method was used to characterize the nonclinical pharmacokinetics and biodistribution profile of T-20 in a series of animal model studies. Results of these studies indicate that following intravenous administration T-20 is cleared from the plasma of rodents and primates by a bi-exponential process. In each species T-20 demonstrates a relatively short alpha phase distribution half-life followed by a prolonged terminal elimination phase half-life. Values determined in rodents for the parameters of Maximum Plasma Concentration (C_{max}) and Volume of Distribution (Vd) were highly predictive for those same parameters determined in primates. Biodistribution studies were performed using a canulated rodent model. These studies indicate that T-20 readily penetrates the lymphatic system and equilibrates with the plasma reservoir of drug within 30 minutes post administration. The T-20 LAB ELISA was evaluated for its use in supporting clinical trials. Studies performed in primates indicate that the assay yields consistent AUC values over time. Studies performed in normal patient plasma indicate that the assay is highly quantitative in that matrix; however, non-uniform recovery of analyte from the plasma of HIV-1 positive patients precludes the use of this method for the determination of clinical pharmacokinetic parameters. To solve this problem, polyclonal rabbit anti-T-20 antibodies were used to develop a sandwich capture ELISA assay (T-20 PcAb Sandwich ELISA). The assay is specific and quantitative for detecting analyte in HIV-1 positive plasma and yields results which are comparable to those obtained with the T-20 LAB ELISA. The T-20 PcAb Sandwich ELISA was used to determine clinical pharmacokinetic parameters from patients who received T-20 by intravenous infusion in an ongoing phase 1/2 multidose, dose escalation clinical trial.

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SYNTHESIS AND ANTI-HBV ACTIVITY OF 2'-FLUORO-β-L-ARABINOFURANOSYL PURINE NUCLEOSIDES

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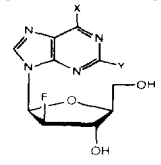
Since the discovery of 2'-fluoro-5-methyl-β-L-arabinofuranosyl uracil (L-FMAU) as a potent anti-HBV and anti-EBV agent, we have studied the structure-activity relationships of 2'-deoxy-2'-fluoro-β-L-arabinofuranosyl pyrimidine nucleosides as anti-HBV agents. Therefore, it is rational to extend this study to the purine nucleoside analogues. Thus 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-L-arabinofuranosyl bromide, which was prepared from L-xylose via a multistep procedure, was coupled with a variety of purine nucleobases.



From this general synthesis, several purine nucleosides containing the 2-deoxy-2-fluoro-β-L-arabinofuranosyl moiety were obtained. The anti-HBV activity and toxicity of the synthesized nucleosides were evaluated in 2.2.15 cells. The adenine (EC_{50} 1.5μM) and the hypoxanthine (EC_{50} 8μM) analogues showed moderate anti-HBV activity. (This research was supported by NIH Grant AI 33655)