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Pharmacokinetics of (-)-2',3'-Dideoxy-5-Fluoro-3'-Thiacytidine [(-)-FTC] and its Metabolites in Rhesus Monkeys. L. E. Moore [1], L. Ni [1], F. D. Boudinot [1], H. M. McClure [2], and R. F. Schinazi [2,3,4]. [1]College of Pharmacy, University of Georgia, Athens, Georgia 30602; [2]Yerkes Regional Primate Research Center, and [3]Department of Pediatrics, Emory University School of Medicine, Emory University, Atlanta, Georgia 30322; and [4]Georgia Research Center for AIDS and HIV Infections, Veterans Affairs Medical Center, Decatur, Georgia, 30033, USA.

(-)-FTC has been shown to have potent antiviral activity against human immunodeficiency virus type 1 and type 2 and hepatitis B virus *in vitro*. The purpose of this study was to characterize the pharmacokinetics of (-)-FTC in monkeys. Rhesus monkeys were administered 200 μCi of radiolabeled (-)-FTC with 33.3 mg/kg of (-)-FTC intravenously. Concentrations of (-)-FTC and its metabolites in serum, urine, and cerebrospinal fluid were determined by HPLC. Pharmacokinetic parameters were generated by compartmental analysis. Concentrations of (-)-FTC in serum declined in a biexponential manner and were well described by a two compartment model. Total clearance of (-)-FTC averaged 0.67 $\pm$ 0.05 L/h/kg (mean $\pm$ SD) and steady-state volume of distribution averaged 1.10 $\pm$ 0.14 L/kg. The terminal phase half-life of (-)-FTC was 1.6 $\pm$ 0.2 h. Renal clearance was the prevalent mode of elimination, with a fraction excreted unchanged in the urine of 63 $\pm$ 7%. Two metabolites of (-)-FTC were identified in serum and urine of monkeys: (-)-FTC-glucuronide and 3'-SO-(-)-FTC. Peak metabolite concentrations were observed within 25 min after drug administration. CSF:serum (-)-FTC concentration ratios were time dependent and no metabolites were detected in CSF. (-)-FTC did not appear to undergo significant cleavage to fluorocytosine. (NIH grants AI-31827 and RR-00165, and the VA).

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Pharmacokinetics of ß-L-2',3'-dideoxy-5-Fluorocytidine in Rhesus Monkeys

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ß-L-2',3'-dideoxy-5-Fluorocytidine (ß-L-FddC), a novel cytidine analog with an unnatural ß-L sugar confirmation, has been demonstrated by our group and others to possess highly selective *in vitro* activity against HIV-I, HIV-II, and HBV. This encouraging *in vitro* antiviral activity prompted our group to assess the pharmacokinetics of ß-L-FddC in rhesus monkeys. Three monkeys were administered a 5 mg/kg intravenous dose of [^{3}H]-ß-L-FddC. Following a 15 week washout period, an equivalent aqueous oral dose was administered. Serum and urine samples were collected at various time points up to 24 hours after dosing and drug levels were quantified by HPLC. Pharmacokinetic parameters were obtained based on a model-independent analysis. After intravenous administration, mean peak serum concentration (C_{MAX}) was 22.4 $\pm$ 4.8 µM. Systemic clearance (CL), apparent volume of distribution (Vd), terminal serum half-life ($T_{1/2}$), and mean residence time (MRT) were 2.4 $\pm$ 0.4 L/h, 44.3 $\pm$ 8.1 L, 12.9 $\pm$ 4.2 h, and 8.5 $\pm$ 2.6 h, respectively. Approximately 46.3 $\pm$ 17.0% of the administered radioactivity was recovered in the urine as the unchanged drug with no metabolites being detected. Oral ß-L-FddC exhibited a C_{MAX} of 3.8 $\pm$ 1.1 µM with a lag time of approximately 1.5 hours and a terminal $T_{1/2}$ of 9.4 h. One monkey in the oral arm of the study had a significant delay in the absorption of the aqueous administered dose, but the AUC was not affected. The absolute bioavailability (F) of the orally administered ß-L-FddC solution ranged from 56 % to 82 %. The encouraging bioavailability of ß-L-FddC in conjunction with its highly selective *in vitro* antiviral activity warrants further investigation of the drug.

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Discovery of Antiviral Agents for Hepatitis C: Development of a High Throughput Assay for the NS3 Nucleoside Triphosphatase. E.M. August, L. Patnaude, D. Davis, J. Cryan, L.-A. Yeh. Phytera, Inc., Worcester, MA, USA.

Recent advances in the molecular biology of the hepatitis C virus (HCV) have resulted in the cloning of several enzymes important in viral replication, and thus may provide suitable targets for drug discovery. These include an RNA-directed RNA polymerase and a metalloprotease. The NS3 protein of HCV possesses 3 distinct enzymatic activities: a serine protease, a nucleoside triphosphatase (ATPase), and an RNA helicase. We previously reported a kinetic assay for the ATPase activity of a soluble, truncated form of the NS3 protein (NS3b) which has been cloned and overexpressed in *E. coli*, as well as a gel shift assay for the helicase activity (Antiviral Res., 30, 25, 1996). To facilitate high throughput screening of Phytera's library of plant cell culture and marine microorganism extracts for anti-NS3b ATPase activity, we have adapted the Fiske-SubbaRow colorimetric assay for inorganic phosphate to a 96-well microtiter plate format and have initiated automated robotic screening. The assay response was linear up to an OD_{850} of 1.0, corresponding to a phosphate concentration of approximately 6.25 µg/mL. The reaction velocity was linear with increasing NS3b up to 25 µg/mL, and for at least 60 minutes at 25° C. The assay was further modified to run fully automated on a Rosys Plato robotic system, handling approximately 1600 samples per day in duplicate. This robotic primary assay is now being used in conjunction with secondary assays (a mammalian ATPase assay to examine specificity and the gel shift assay to evaluate activity against the helicase) to discover potential anti-HCV agents. High-throughput assays for the RNA helicase, as well as other HCV enzymatic targets, are currently under development.

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Development of a Hepatitis C Virus RNA Helicase High Throughput Assay

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An assay has been developed to detect inhibitors of the RNA helicase activity of Hepatitis C virus. Double stranded RNA substrate is unwound by the action of the enzyme and this signal increase assay measures the capture of the released radiolabelled single strand by a complementary biotinylated oligomer bound to a streptavidin coated NEN FlashPlate. A standard dsRNA substrate is used in the assay. Complementary single stranded RNAs are prepared by *in vitro* transcription, and one strand is labelled with [^{32}P]. Following gel purification the ssRNAs are hybridized to form the dsRNA substrate and re-purified. The assay enzyme consists of the full NS3 protein complexed with 4A which contains the protease domain in addition to the helicase and NTPase domains. A His tag is fused to the amino terminus of the protein, allowing affinity purification over a Ni-NTA column. The enzyme is expressed and purified from baculovirus-infected Sf9 cell lysates. FlashPlates are initially treated with the biotinylated capture oligomer. Substrate and enzyme are incubated in the FlashPlate wells. Action of the enzyme results in a signal increase which can be prevented by the presence of an inhibitor. This assay format is applicable to dsNAs and helicases from other sources.