27

Cellular Pharmacology of ß - L - Dideoxycytidine, ß - L - 2', 3' Dideoxy - 5 - Fluorocytidine, and ß - D - 2', 3' Dideoxy - 5 - Fluorocytidine. L.T. Martin[1], R.F. Schinazi[2], G. Gosselin[3], J.-L. Imbach[3], and J.-P. Sommadossi[1]. University of Alabama at Birmingham, Birmingham, AL 35294 USA;[1] VA Medical Center / Emory University, Decatur, GA 30033 USA;[2] University of Montpellier II, 34095 Montpellier, France.[3]

Over the last years, unnatural L - configured nucleosides have been demonstrated to provide an increased selectivity as compared to their corresponding D enantiomers in the search for new antiviral therapies against HIV and HBV infections. In the present study, we have investigated the cellular pharmacology of the ß - L enantiomer of 2',3' - dideoxycytidine (L-ddC) and its 5 - fluoro derivative (L-FddC) and compared it to corresponding ß - D - 2',3' - dideoxy - 5 - flourocytidine (D-FddC). These compounds were demonstrated by our group to be active against HIV and HBV replication *in vitro* (Gosselin et. al., AAC 1994, 38, 1292 - 1297 and Schinazi et. al., AAC 1994, 38, 2172 - 2174). L-FddC (10 µM) was rapidly phosphorylated in HepG2 cells to its 5'- mono -, di -, and triphosphate derivatives with intracellular levels of 7.7, 19.9, and 29.9 pmol/10^6 cells, respectively, after 72 hours. The 5' - phosphorylated derivatives of D-FddC achieved equal or lower levels of 7.8, 8.6, and 2.6 pmol/10^6 cells under the same conditions. A choline derivative, L-FddCDP-choline, was detected and achieved a concentration of 9.5 pmol/10^6 cells within 72 hours. Both choline and ethanolamine derivatives were detected in D-FddC treated cells and concencentrations of 15.7 and 13.4 pmol/10^6 cells were determined for each derivative, respectively. The intracellular half - life of L-FddCTP was assessed in HepG2 cells following a 48 hour exposure to 10 µM L-FddC. L-FddCTP underwent a two phase process of elimination with an initial $T_{1/2}$ of 0.25 hours for the first two hours followed by a longer $T_{1/2}$ of 10.5 hours with levels of 4.0 pmol/10^6 cells being still detectable 24 hours following drug removal. L-ddC, at a concentration of 1 µM, was also rapidly phosphorylated in HepG2 cells to its 5' - mono -, di -, and triphosphate derivatives with intracellular level reaching 0.3, 0.7, and 4.8 pmol/10^6 cells, respectively, after 72 hours. L-ddCDP-choline was also detected and accounted for a concentration of 3.3 pmol/10^6 cells at 72 hours. These results indicate that L-FddC is preferentially phosphorylated to its active 5' - triphosphate as compared to D-FddC in HepG2 cells. Further studies are in progress to detail the intracellular disposition of these novel nucleosides

28

Selective Liver Targeting of a Derivatized Antiviral Nucleoside Analogue by Recombinant Chylomicrons. P.C.N. Rensen, M.C.M. van Dijk, E.C. Havenaar, M.K. Bijsterbosch, J.K. Kruijt, and T.J.C. van Berkel. Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, University of Leiden, Sylvius Laboratories, P.O. Box 9503, 2300 RA Leiden. The Netherlands.

Hepatitis B virus (HBV) infection is currently the most important chronic virus infection, but no safe and effective therapy is available at present. Clinical exploration of promising antiviral agents, such as nucleoside analogues, is hampered because their aspecific body distribution leads to significant toxic side-effects. Chylomicrons transport lipids from the intestine to the liver via apolipoprotein (apo)E-specific receptors on parenchymal cells, the main site of infection and replication of HBV, and could therefore well serve as drug carriers. Since their endogenous nature hampers their pharmaceutical application, we constructed recombinant (*rec*) chylomicrons from commercially available lipids and human *rec*-apoE, and evaluated their use in liver targeting of a dioleoyl derivative of iododeoxyuridine ($IDU(OI)_2$). Emulsions were prepared by sonication, and resembled chylomicrons with respect to size (82.2±2.9 nm) and composition (78.1±0.6% triolein, 14.4±0.5% egg yolk phosphatidylcholine, 1.0±0.3% lysophosphatidylcholine, 4.2±0.3% cholesteryl oleate, and 2.4±0.1% cholesterol, w/w). After injection of [^{3}H]cholesteryl oleate labeled emulsions into fasted rats, a serum half-life of 15 min was observed accompanied by a liver uptake reaching 36% of the injected dose at 30 min after injection. Prior enrichment with *rec*-apoE (68±12 molecules per particle) led to a faster serum decay ($t_{½}$=5 min) and an increased liver uptake of the emulsion (70% at 30 min after injection). Isolation of the various liver cell types at 30 min after injection indicated that parenchymal cells were mainly responsible for emulsion uptake (>80% of the total liver association), similarly as observed with chylomicrons. Injection of [^{3}H]$IDU(OI)_2$ incorporated into the apoE-enriched emulsion (90-130 molecules per particle), resulted in similar *in vivo* kinetics of prodrug and emulsion core label indicating that both were removed from the blood circulation as a unity. A 39-fold increased uptake of the prodrug by the liver parenchymal cells was observed as compared to that of the free drug, attaining potentially effective intracellular concentrations of at least 50 nM. We anticipate that our findings could renew interest in anti-HBV nucleoside analogues with associated severe extrahepatic side-effects, and might lead to a new and more effective strategy to fight hepatitis B and HBV-induced diseases.