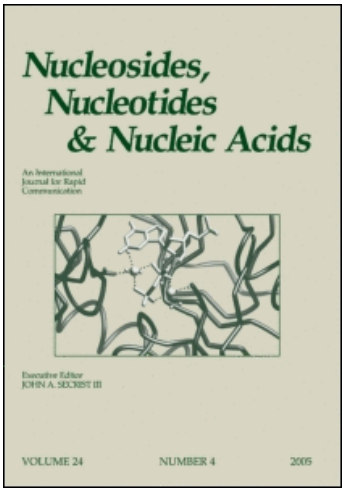

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Studies of the Pharmacokinetics and Toxicology of 2',3'-Dideoxy-β-L-5-fluorocytidine (β-L-FddC) and 2',3'-Dideoxy-β-L-cytidine (β-L-ddC) *In Vivo*; and Synthesis and Antiviral Evaluations of 2',3'-Dideoxy-β-L-5-azacytidine

Tai-Shun Lin[a]; Xin Guo[a]; Mei-Zhen Luo[a]; Mao-Chin Liu[a]; Yong-Lian Zhu[a]; Ginger E. Dutschman[a]; S. Balakrishna Pai[a]; Mao-Mi Li[b]; Yung-Chi Cheng[a]
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STUDIES OF THE PHARMACOKINETICS AND TOXICOLOGY OF 2',3'-DIDEOXY-β-L-5-FLUOROCYTIDINE (β-L-FddC) AND 2',3'-DIDEOXY-β-L-CYTIDINE (β-L-ddC) *IN VIVO*; AND SYNTHESIS AND ANTIVIRAL EVALUATIONS OF 2',3'-DIDEOXY-β-L-5-AZACYTIDINE

Tai-Shun Lin,* Xin Guo, Mei-Zhen Luo, Mao-Chin Liu, Yong-Lian Zhu,
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Abstract: The pharmacokinetics and toxicology of 2',3'-dideoxy-β-L-5-fluorocytidine (β-L-FddC) and 2',3'-dideoxy-β-L-cytidine (β-L-ddC) in mice was investigated. In addition, 2',3'-dideoxy-β-L-5-azacytidine (β-L-5-aza-ddC) and its α-L-anomer (α-L-5-aza-ddC) were synthesized by coupling the silylated 5-azacytosine derivative with 1-*O*-acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-L-ribofuranose, followed by separation of the α- and β-anomers and were evaluated *in vitro* against HBV and HIV. β-L-5-aza-ddC was found to show significant anti-HBV activity at approximately the same level as 2',3'-dideoxy-β-D-cytidine (ddC), which is a known anti-HBV agent. β-L-5-aza-ddC was not cytotoxic to L1210, P388, S-180, and CCRF-CEM cells up to a concentration of 100 μM. Conversely, the α-L-anomer was not active against HBV at the same concentration.

Recently, the synthesis and antiviral activity of 2',3'-dideoxy-β-L-5-fluorocytidine (β-L-FddC) and 2',3'-dideoxy-β-L-cytidine (β-L-ddC) were reported by our laboratory[1-3] and by others.[4-6] β-L-FddC and β-L-ddC were found to have potent anti-HBV and anti-HIV activity *in vitro*. The pharmacokinetics and toxicology of these two compounds were investigated in mice. The study involved measuring the blood plasma concentration levels of β-L-FddC and β-L-ddC as a function of time after oral administration. The plasma concentrations of β-L-FddC and β-L-ddC reached their maximum levels ~1800 times higher than that of their ED_{50} values (0.01 μM)[2] for HBV infection at approximately one hour after oral administration of 50 mg/Kg of each compound. β-L-FddC was found to maintain the high plasma concentration for a longer period than β-L-ddC. In determining the relative toxicity of β-L-FddC and β-L-ddC, five groups of six randomly selected mice were administered orally 50 mg/Kg of either β-L-FddC or β-L-ddC twice a day for 30 days. No weight-loss toxicity was observed. At the end of the experiments, autopsies revealed no

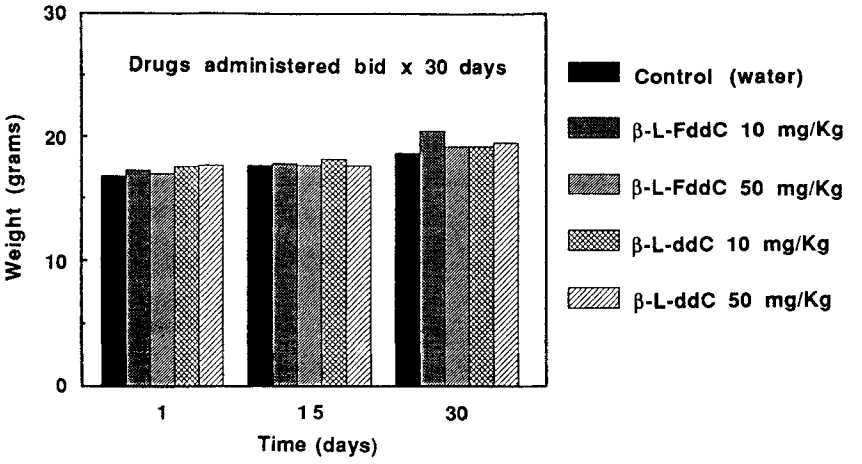


Figure 1. Toxicity of β-L-FddC and β-L-ddC in female BDF_1 mice.

brain, heart, liver, bone-marrow, pancreas, kidney, or muscle abnormalities. These findings are shown in the Figures 1 and 2.

Groups of six randomly selected mice were orally administered β-L-FddC or β-L-ddC at 10 or 50 mg/Kg dissolved in water or water alone, twice a day for thirty days. During this period they were observed for general health and were weighed daily as an index of toxicity. The data points above (Figure 1) indicate no weight-loss toxicity and show only a slight weight gain as the animals matured. Three mice from each group were terminated on the thirtieth day. Tissues and blood were taken at this time for further studies. No abnormalities were observed in the paraffin sections of tissues from these mice, which include brain, heart, liver, bone-marrow, pancreas, kidney, and muscle. Plasma from these mice was also collected and used for clinical chemistry studies. The other three mice from each group were used for the pharmacokinetic studies.

Plasma samples from groups of three mice treated with 10 mg/Kg or 50 mg/Kg of β-L-FddC or β-L-ddC were pooled and used for clinical chemistry studies including creatinine and bilirubin levels, as well as the activity of the liver enzymes serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalic transaminase (SGOT), lactate dehydrogenase (LDH), and alkaline phosphatase. While the levels determined in the tests sometimes fall outside the standard human values, there appears to be no significant differences between the mice control and treated groups, as shown in Figure 2.

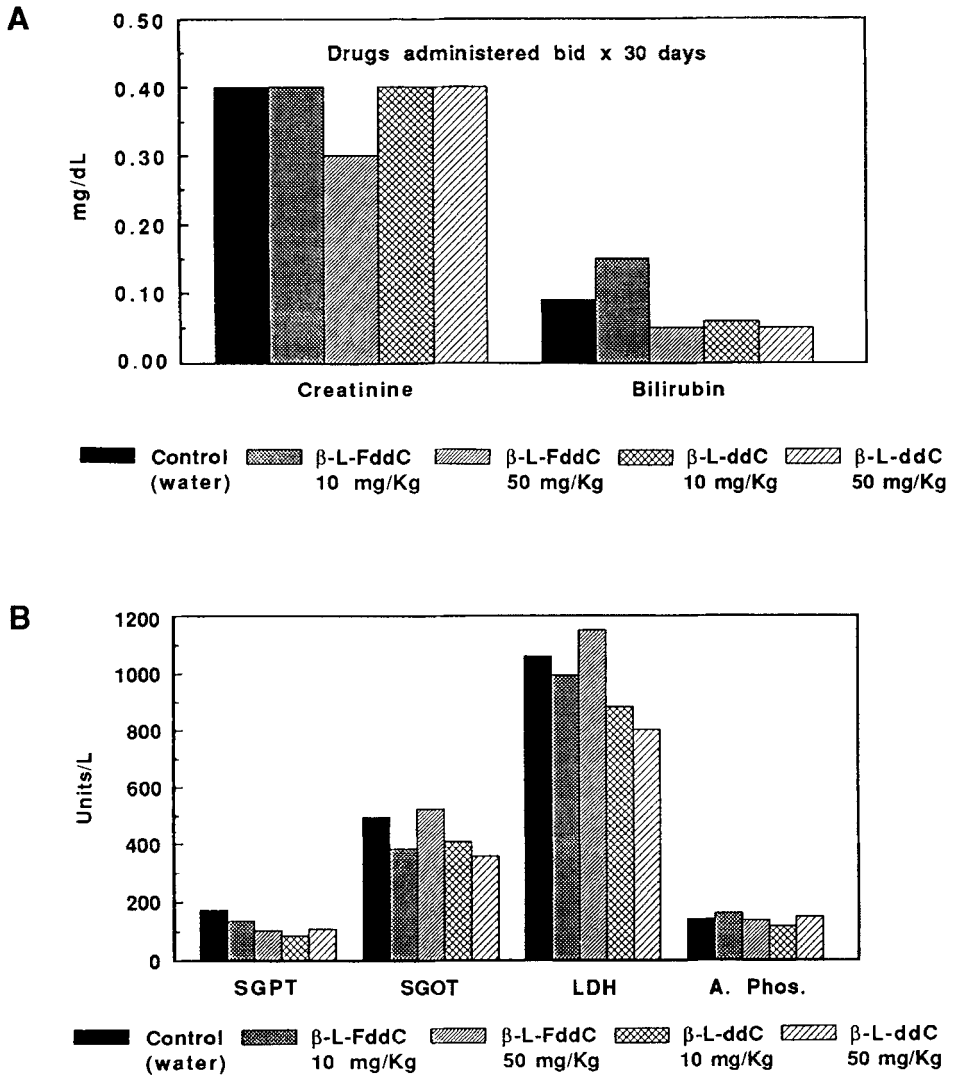


Figure 2: A and B. Clinical chemistry studies of pooled plasma from mice treated with β-L-FddC or β-L-ddC.

β-L-ddC was administered orally into female BDF_1 mice at the concentrations as previously indicated. Each mouse was bled from the retro-orbital sinus, using a heparinized capillary tube. The two groups each represent data from three mice that were treated twice a day for 30 days. The animals were given a final dosing, then the first mouse in each group was bled at 2 and 5 min, the second at 15 and 30 min, and the third at 1 and 2 h. The plasma from each sample was removed after centrifugation and deproteinized by the addition of one half volume of 45% trichloroacetic acid. The supernatants were neutralized by extraction with a mixture of trioctylamine-freon. The samples were then quantitated using HPLC with a SCX column and 0.1 M KPO_4 pH 9 solvent system. Concentrations were calculated by comparison with known amounts of β-L-ddC. The data in Figure 3 suggests that the drug effectively enters the blood after oral administration. It is noteworthy that the ED_{50} concentration for HBV infections was previously determined to be 0.01 μM for both β-L-FddC and β-L-ddC.[2] In the case of β-L-ddC, plasma levels reached their maximum (~800 and 1800 times, respectively, the ED_{50} concentration for HBV infections) at about 30 min after oral administration.

β-L-FddC was given orally to female BDF_1 mice at the concentrations indicated (Figure 4). Each mouse was bled from the retro-orbital sinus, using a heparinized capillary tube. The 50 mg/Kg x 1 line is comprised of data from two mice; one bled at 15 and 30 min and the other at 1 and 3 h post injection. The two, 30-day groups represent data from three mice that were treated twice a day for 30 days. The animals were given a final dose, then the first mouse in each group was bled at 2 and 5 min, the second at 15 and 30 min, and the third at 1 and 2 h. The plasma from each sample was removed after centrifugation and deproteinized by the addition of one half volume of 45% trichloroacetic acid. The supernatants were neutralized by extraction with a mixture of trioctylamine-freon. The samples were then quantitated using HPLC with a C_{18} reverse phase column and 0.03 N acetic acid/10% methanol solvent system. Concentrations were calculated by comparison with known amounts of β-L-FddC. In the case of β-L-FddC, plasma levels reached their maximum (~500 and 1800 times, respectively, the ED_{50} concentration for HBV infections) at about 30 min after oral administration. β-L-FddC exhibited a greater area under the curve suggesting that plasma concentration levels were higher for a longer period of time with β-L-FddC compared to β-L-ddC.

These experiments suggest that β-L-FddC and β-L-ddC effectively enter the blood after oral administration and attain concentrations of drug which would be effective for treating HBV infections in humans. Moreover, the data also indicate that there is no significant difference in the clearance rate of β-L-FddC administered once or twice a day for 30 davs.

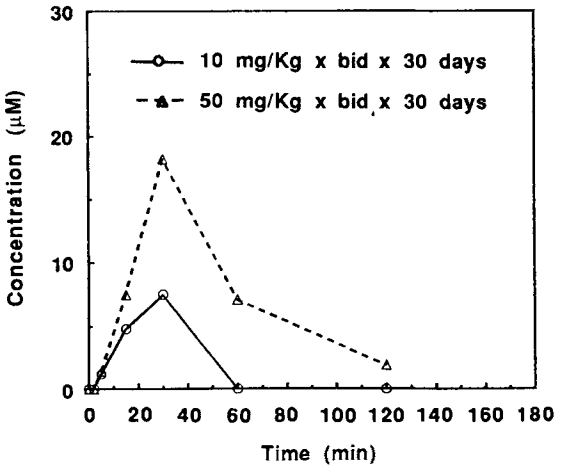


Figure 3. β-L-ddC in mouse plasma after oral administration.

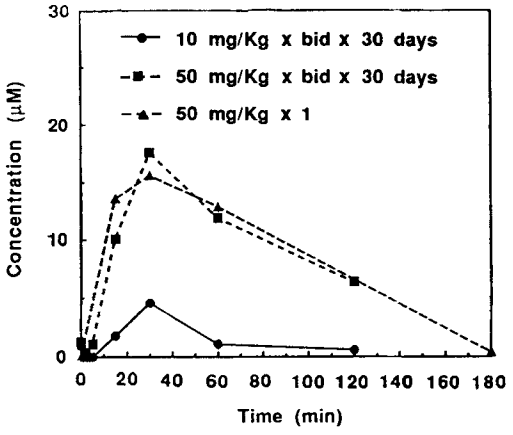


Figure 4. β-L-FddC in mouse plasma after oral administration.

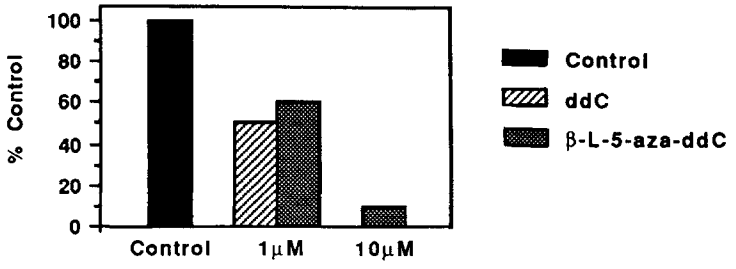
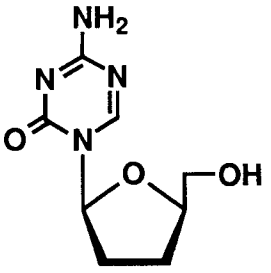


Figure 5. Antiviral activity of β-L-5-aza-ddC and ddC *in vitro* against HBV.

In addition, a new compound 2',3'-dideoxy-β-L-5-azacytidine (β-L-5-aza-ddC) and its α-L-anomer (α-L-5-aza-ddC) were synthesized by coupling of the silylated 5-azacytosine derivative with 1-*O*-acetyl-5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-L-ribofuranose[2] in the presence of ethylaluminum dichloride ($EtAlCl_2$) in CH_2Cl_2, followed by separation of the α- and β-anomers by silica column chromatography and removal of the 5'-blocking group. These compounds were evaluated *in vitro* against HIV-1 and HBV. β-L-5-aza-ddC was found to demonstrate significant anti-HBV activity at approximately the same level as 2',3'-dideoxy-β-D-cytidine (ddC), which is a known anti-HBV agent. At 1 μM, ddC and β-L-5-aza-ddC inhibited the growth of HBV by 40 and 50%, respectively. At 10 μM, β-L-5-aza-ddC inhibited the growth of HBV by 90% (Figure 5). Also, β-L-5-aza-ddC was not cytotoxic to L1210, P388, S-180, and CCRF-CEM cells up to a concentration of 100 μM. Conversely, the α-L-anomer was devoid of any anti-HBV activity.

The structure, physical, and spectroscopic data of β-L-5-aza-ddC are as follows:



β-L-5-aza-ddC

Mp 260 °C (dec); TLC, R_f 0.56 (CH_2Cl_2/MeOH), 6:1, v/v); $[\alpha]_D^{25}$ -45 ° (*c* = 0.1 MeOH); UV (MeOH) λ_{max} 246 nm (ε 5178), λ_{min} 232 nm; (0.01 N HCl) λ_{max} 254 nm (ε 4499), λ_{min} 230 nm; (0.01 N NaOH) λ_{max} 246 nm (ε 5602), λ_{min} 236 nm; ^1H NMR (Me_2SO-d_6) δ 1.80-2.33 (m, 4H, 2'-H and 3'-H), 3.48-3.52 (m, 1H, 5'-H_A), 3.70-3.76 (m, 1H, 5'-H_B), 4.00-4.07 (m, 1H, 4'-H), 5.09 (t, 1H, 5'-OH, D_2O exchangeable), 5.83-5.87 (m, 1H, 1'-H), 7.39-7.41 (br s, 2H, 4-NH_2, D_2O exchangeable), 8.62 (s, 1H, 6H).

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