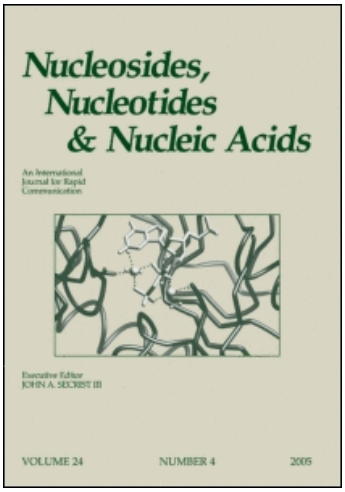

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A Dihydropyridine Carrier System for Delivery of 2',3'-Dideoxycytidine (DDC) to the Brain

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A DIHYDROPYRIDINE CARRIER SYSTEM FOR DELIVERY OF 2',3'- DIDEOXYCYTIDINE (DDC) TO THE BRAIN

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Abstract: The present study extends the dihdropyridine ⇌ pyridinium salt redox system to the delivery and sustained release of 2',3'-dideoxycytidine (DDC) to the brains of mice in a continuing search for agents that may prove effective in reversing complicating neurological disorders of AIDS.

Introduction

Neurological disorders are common and important causes of morbidity among patients in advanced stages of infection with human immunodeficiency virus (HIV)[1]. For example, there is evidence that the complicating neurological syndrome, AIDS dementia complex, is caused partially or wholly by direct HIV-infection of the brain[2-5]. The virus is transported to the central nervous system (CNS) by infected macrophages/monocytes where it serves as a major reservoir for infection[6]. Because HIV exhibits tissue tropism for the CNS and macrophages/monocytes in addition to T lymphocytes[4], the virus is able to cross the brain-capillary wall, i.e., the blood-brain-barrier (BBB). However, the mechanism of HIV-induced CNS-dysfunction remains unclear. Nonetheless, there is an important need for antiviral agents that can penetrate the BBB.

3'-Azido-3'-deoxythymidine (AZT, Zidovudine), like thymidine, readily crosses the human blood- cerebral spinal fluid (CSF)-barrier and distributes in the CSF.[7] This finding is consistent with observations that AZT ameliorates the condition of AIDS dementia in some patients.[8] On the other hand, the clinical data appear to be at odds with the results of a study which showed that, in carotid artery-injected rats, AZT is not measurably transported through the BBB[9]. Consequently, the distribution of AZT into brain interstitial fluid, as suggested by Terasaki and Pardridge[9], may be minimal.

Clearly, effective chemotherapeutic eradication of HIV requires that the antiviral agent cross the BBB. Recognition of the challenge prompted efforts[10] of brain- specific and sustained delivery of AZT by means of a redox system based on a dihydropyridine-pyridinium salt- interconversion. This strategy has been utilized by Bodor and coworkers[11]for delivery of a wide spectrum of drugs to the CNS. The approach was subsequently extended to the successful chemical delivery of 3'-azido-2',3'-dideoxyuridine (AZDU)[1b] and 2',3'-didehydro-3'-deoxythymidine (D4T)[1c] to the brain of mice.

DDC (**1**), on a mole for mole basis, is about ten-fold more potent than AZT at killing HIV[12] but the pharmacokinetic value of CSF-penetration for AZT is threefold higher than that obtained for DDC[13]. Moreover, DDC like AZT, is not measurably transported through the BBB[9]. These observations provided the rationale to extend the redox carrier system described above to delivery of DDC to the brain[14].

Chemistry

The synthesis of the drug carrier system **5** (Figure I) proceeded from **1**[15] which was first protected in the form of a 4-dimethylaminomethylene derivative, **2**. The latter was successively esterified with nicotinic acid and 1,3-dicyclohexylcarbodiimde, de-protected, and the product (**3**), then, quaternized to **4** with methyl iodide in acetone. Without further purification, **4** was reduced with sodium dithionite under nitrogen to give **5** as a yellow, hygroscopic solid (11% overall yield) which afforded a satisfactory elemental analysis. Moreover, the ^{1}HNMR characteristics of 5 are in agreement with corresponding data reported previously for other 5' - O-

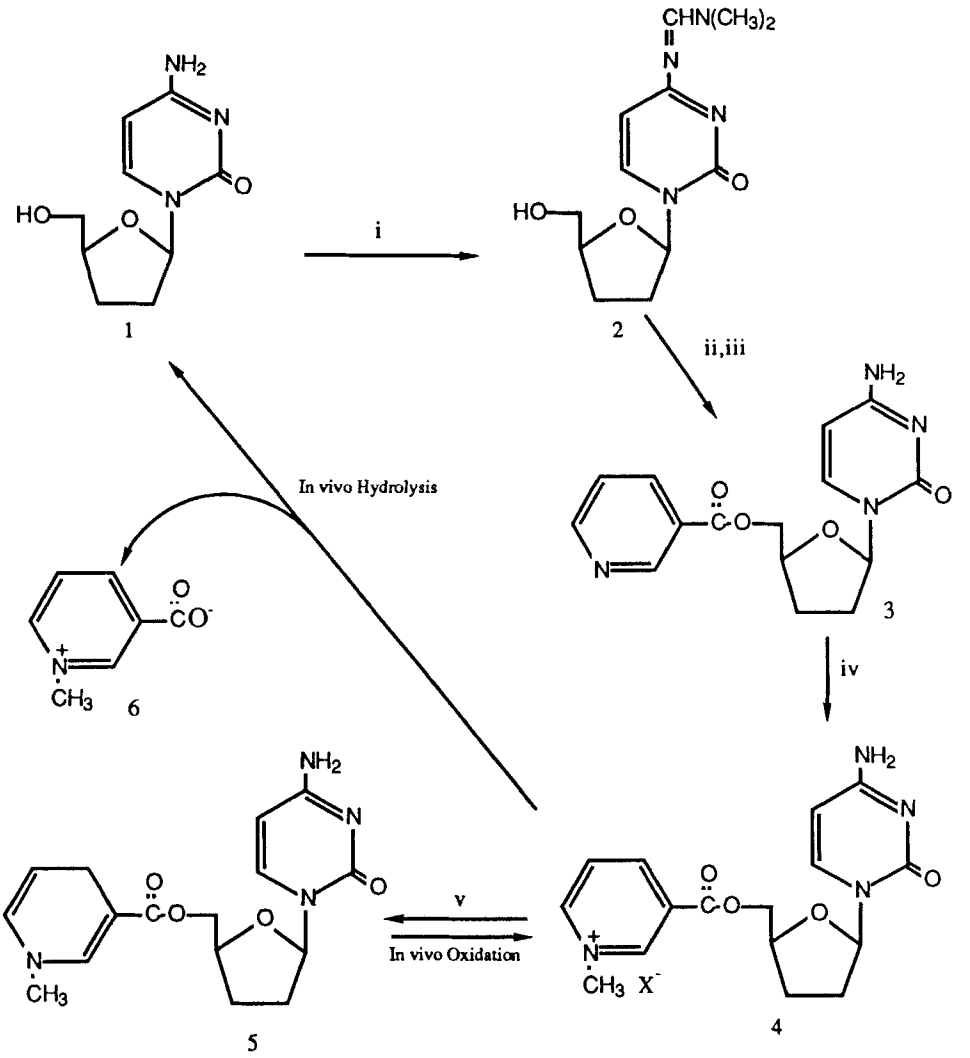

Figure I

Reagents:

i, Dimethyl formamide dimethyl acetal; ii, DCC, DMAP, nicotinic acid, DMF;

iii, n-butanol, acetic acid,water; iv, MeI/acetone; v, $Na_2S_2O_4$, aqueous methanol.

dihydronicotinoyl derivatives of 2',3,dideoxynucleosides[1a,b,10a]. For example, this same 1,4 dihydro-1-methyl-3-carbonyl moiety attached to D4T shows absorptions at 2.902 (N-CH3 + H4"), 4.70 (H5") and 5.82 (H6") whereas the same substituent in **5** displays corresponding chemical shifts at 2.735 (N-CH3 + H4"), 4.62 (H5") and 5.63 (H6"). There was no evidence in the ^{1}HNMR spectrum of **5** of either isomerization or decomposition after storage at low temperature. However, tlc of a sample of **5** held at room temperature revealed that the product had been partially converted to the parent nucleoside along with an unknown, amber colored, more polar product. For this reason **5** was stored at -20°C and examined by HPLC just prior to use in the biochemical and pharmacological studies.

It is interesting to note that, in contrast to **5**, the corresponding derivatives of AZT[1a,10a] and D4T[1c] are both relatively more stable and, accordingly, exhibit better shelf-life. Surprisingly, the dihydropyridine derivative of AZDU is also sensitive to air-oxidation and hydrolysis.[1a] . The reason(s) for these differences among the dihydropyridine derivatives are not apparent.

Attempts to extend the synthetic methodology described above to the preparation of the corresponding 5'-O-dihydronicotinate ester of 2',3'-dideoxyinosine (DDI) were unsuccessful. Treatment of the latter with either nicotinic acid or nicotinic anhydride and 1,3-dicyclohexylcarbodiimide, or 1-methylnicotinic acid (trigonelline) anhydride in the presence of DMAP[16] led, in each case, to cleavage of the glycosyl bond of DDI.

Biochemistry and Pharmacology

Pseudo-first-order rate constants and half-lives for the oxidation of the 1,4-dihydropyridine ester **5** by hydrogen peroxide and different biological media are compared in Table 1 with corresponding constants obtained for the same ester of 2',3'-didehydro-3'-deoxythymidine (**7**) in our earlier study[1c].

It was observed, in accord with observations noted above, that the rates of oxidation of **5** exceed those of **7** in chemical as well as in biological media. Rate constants for the oxidation of **5** with mouse liver ($k=21 \times 10^{-5}\ s^{-1}$) and brain tissues ($18 \times 10^{-5}\ s^{-1}$) were 2.9- and

Table 1. Kinetics of in Vitro Biological and H_2O_2 Oxidations of the Dihydropyridine Esters of DDC (**5**) and D4T (**7**) to Quaternary Salts.

medium	K, s^{-1} x 10^{-5}		$t_{1/2}$ min	
	5	**7**	**5**	**7**
hydrogen peroxide	140 ± 12	55 ± 3.2	8.2 ± 0.7	21 ± 1.3
human plasma	7.2 ± 0.85	3.54 ± 0.11	160 ± 15	325 ± 10
mouse brain homog.	18 ± 1.6	8.85 ± 0.34	65 ± 4.6	130 ± 5
mouse liver homog.	21 ± 1.7	9.20 ± 0.37	55 ± 3.8	125 ± 5

2.5-fold higher, respectively, than in human plasma (7.2 x 10^{-5} s^{-1}). The ratios compare favorably with those obtained for the oxidation of **7**, which were 2.6- and 2.5- fold higher with mouse liver and brain tissues, respectively than in human plasma.

There was no indication of significant hydrolysis of either ester during the course of the oxidations. However, it should be noted that the in vitro oxidations, in contrast to the in vivo studies (see Table 2), were carried out over short periods of time and in dilute solutions which might be deficient in important cofactors necessary for effective hydrolysis.

The elucidation of the mechanism(s) of the biochemical oxidations was beyond the scope of the present work. Nonetheless, the oxidation-studies, together with an enhanced partition coefficient (octanol/ water) observed for **5** (Log P - 0.3±0.01) compared to **1** (Log P - 1.15 ± 0.03), predict for an accumulation of a quaternary derivative in the brain with a relatively slow release of **1** from the oxidized product by enzymic and/or chemical hydrolysis.

Compound **5** was injected in C3H female mice at a dose of 25 mg/kg and following sacrifice, brain and liver tissues were obtained 1, 4 and 24h later. Trigonelline (1-methylnicotinic acid, **6**) and **1**,

Table 2. Comparison of the Distribution of DDC (**1**) and Trigonelline (**6**) in Liver and Brain after administration of Nucleoside-Carrier System **5** to Mice[a].

	1		**6**	
time(h)	**liver**	**brain**	**liver**	**brain**
1	0.716 ± 0.185	0.712 ± 0.236	0.68 ± 0.23	0.65 ± 0.19
4	0.693 ± 0.175	0.648 ± 0.190	0.56 ± 0.21	0.52 ± 0.16
24	0.299 ± 0.093	0.280 ± 0.104	0.22 ± 0.09	0.20 ± 0.08

[a] Compound **5** was injected into the tail vein of C3H female mice at a dose of 25 mg/kg in water. Animals were sacrificed at the indicated intervals, the organs were taken and homogenized in 50% methanol in preparation for HPLC analysis (See Experimental). Three mice were used to establish each time-point. Units equal μg/g wet tissue. [b]The noise level in the region at which **6** elutes made it difficult to get more precise results.

which showed retention times of 3.4 and 4.7 min, respectively, on reverse phase HPLC (λ 295nm; 20 mM phosphate buffer, pH 6.2: acetonitrile) were readily identified in both brain and liver tissue homogenates, prepared at the same intervals. However, because of spectral background-interference, none of the precursory pyridinium ester **4**, which showed a retention time of 5.4 min on HPLC, could be detected in the homogenates. The interference derives from a peak at 5.0-5.5 min from components which absorb at 260-290 nm and are present in control and tissue homogenates.

Table 2 shows that **1** is released in the brain within 1h, at which time it attains a level essentially equal to that detected in liver. Moreover, the levels of **1** in both organs remain virtually unchanged for at least 4h, then decline to approximately one-third of initial values, but only after 24h. The fact that levels of **6** equal to those of **1** were found in the brain homogenates indicates that the 1,4-

Table 3. Distribution of 2',3'-Dideoxycytidine (**1**) in Plasma, Liver and Brain.

Time (hr.)	Liver	Brain
4	0.35 ± 0.09	0.22 ± 0.04
24	0.20 ± 0.05	0.15 ± 0.03

dihdropyridine ester **5** penetrates the BBB where it is oxidized to a quaternary salt (**4**). The latter, because of its polar character, is much less likely to exit the BBB and very probably, not by the same route by which **5** gained entry. Rather, **4** serves, instead, as a source of the observed products of hydrolysis, **1** and **6**.

In support of **5** as the proposed chemical delivery system for **1**, it was found (Table 3) that mice receiving 25 mg/kg of **1**, containing 100 μCi of 2',3'-^{3}H-DDC, show molar concentrations of drug in both brain and liver tissues after 4h which are approximately two-fold less than that delivered via **5**.

Female C3H mice received, via tail vein injection, 25 mg/kg of **1** containing 100 μCi of 2',3'-^{3}H DDC. Three mice were used to establish each level. Units equal μg/g of wet tissue or μg/mL of blood.

The conversion of DDC to a dihydropyridine derivative (**5**) , is superior to simple ester- or amide-type prodrugs in that, according to Bodor[11], the agent (**1**) is delivered specifically to the brain, while maintaining a lower peripheral concentration. Centrally, the efficacy of **1** should be enhanced with concurrent reduction in peripheral toxicity. Indeed, the chemical delivery system **5** merits evaluation in alternating and intermittent administrations[17,18] with the corresponding dihydropyridine ester of AZT as effective pharmaceuticals for an improved control of HIV.

Experimental Section

General Methods: Melting points were obtained on a Thomas-Hoover capillary melting point apparatus. Elemental analyses were

performed by M-H-W Laboratories, Phoenix, AZ. ^{1}HNMR spectra were obtained with a Nicolet QE 300 FT spectrometer. Electron-impact mass spectra (EI-MS) were run with a Kratos MS80 RFA high resolution instrument. UV spectra were obtained with a Perkin-Elmer Lambda 5 spectrophotometer. Partition coefficients were determined in octanol-water according to the method described by Kessel[19]. HPLC was performed with a Bondapak C_{18} reverse-phase column (5 x 100 mm) with an initial mobile phase of 20mm phospate buffer, pH 6.2 (50%)- CH_3CN (50%), which was increased to 100% CH_3CN in 10 min. 2',3'-^{3}H-DDC (47 Ci/mmol) was obtained from Moravek Biochemicals, Brea, CA .

5'-O-(1,4-Dihydro-1-methyl-3-pyridinylcarbonyl)-2',3,dideoxycytidine (5). 2',3'- Dideoxycytidine[18](1g, 4.74 mmol), dissolved in an excess of dimethylformamide dimethyl acetal, was refluxed for 2 h. The solution was concentrated in vacuo, the residue was dissolved in ethanol and filtered through a plug of silica gel. The solution was concentrated in vacuo and used as such in the next step. The latter (**2**) was dissolved in DMF (10 ml), nicotinic acid (600 mg, 5.0 mmol), 1,3-dicyclohexylcarbodiimide (1.03 g, 5.0 mmol), and 4-(dimethylamino) pyridine (60 mg, 0.5 mmol) were added and the mixture was stirred for 24 h. The resulting cloudy solution was filtered, concentrated in vacuo and then chromatographed on silica gel with ethyl acetate: ethanol (8 : 2).The ester (**3**) was deprotected by stirring it at room temperature for 0.5h with a mixture of n-butanol : acetic acid : H_2O (5:3:2). The solution was then concentrated in vacuo and purified by column chromatography on silica gel with ethyl acetate: ethanol (8 : 2) to produce 0.55g of a solid which was homogeneous by TLC (8:2), mp 164-172 ^oC (dec.). Attempts to obtain an analytical sample of **3** by crystallization failed; 1H-NMR (DMSO-d6-partial) showed the product to be 92-95% pure: 4.55 (m, 2H, H5'), 7.43 (m, 1H, H5"-pyridine), 8.00 (m, 1H, H4"- pyridine), 8.62 (d,1H,H6"-pyridine), 8.942 (s, 1H, H2"-pyridine).

To a solution of impure 5'-0-(3'-pyridinylcarbonyl)-2',3'-dideoxycytidine (**3**) (0.5g) in 200 mL of dried (molecular sieves, 4Å) acetone was added 2.0 mL of methyl iodide and the solution was refluxed for 18h. The mixture was evaporated to dryness in vacuo

and the residue, after trituration with acetone, was dried in vacuo to give 0.6 g of a yellow-reddish solid (**4**) mp 150°C (dec) that displayed the characteristic nicotinyl absorptions in a ^{1}HNMR (DMSO-d6) spectrum: 8.1 (t,1H, H5"), 8.7 (d,1H, 4"), 9.0 (d,1H, H6"), 9.5 (S,1H, H2"), but showed two CH3 absorptions at 4.4 - 4.5 ppm. Attempts to purify this material were unsuccessful and the sample was used as such in the reduction step.

To a solution of 0.6g of the impure salt (**4**) in 10% aqueous methanol, through which a stream of N2 had previously been passed for 5 min., was added, sequentially and in the course of 10 min. under N_2, 0.1g of sodium bicarbonate and 1.2g of sodium dithionite. After 20 min. the resulting solid was collected and purified by column chromatography on silica gel. using ethyl acetate:ethanol (8:2) as the eluent. The eluate was evaporated to dryness to give 0.15g of **5** as a hygroscopic, yellow solid, mp 150-2°C (dec); UV λmax (EtOH): 352nm (ε 6,583), 255nm (ε 8,220); 1H-NMR: 2.735 (br. s, 5H, N-CH3 + H4"), 3.37 (m, 2H, H2', H3'), 3.51 (m, 2H, H5'), 3.97 (m, 1H, H4'), 4.62 (m, 1H, H5"), 5.63 (d, 1H, H6"), 5.89 (m, 1H, H1'), 7.12 (s, 1H, H2"), 7.5 (m, 2H, H5, H6). Anal. Calcd for $C_{16}H_{20}N_4O_4$: C, 57.83; H, 6.02; N, 16.87. Found: C, 58.02; H, 5.80; N,16.70. The product is readily converted to a mixture of **1** and a dark yellow material on storage at room temperature.

Oxidations of 4. In vitro oxidations in biological media and by hydrogen peroxide were carried out as described in our previous study[1a].

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REFERENCES

(1) For a comprehensive list of references to neurological complications related to AIDS see the citations in: (a) Fauci, A.S.

Science **1988, 239**, 617-622; (b) Chu, C.K.; Bhadti, V.S.; Doshi, K.J.; Gallo, J.M.; Boudinot, F.D.; Schinazi, R.F. J. Med. Chem **1990, 33**, 2188-2192; (c) Palomino, E.; Kessel, D.; Horwitz, J.P. J. Med. Chem. **1989, 32**, 622-625.

(2) Shaw, G.M.; Harper, M.E.; Hahn, B.H.; Epstein, L.G.; Gajdusek, D.C.; Price, R.W.; Navia, B.A.; Petito, C.K.; O'Hara, C.J.Groopman, J.E.; Cho, E.-S.; Oleske, J.M.; Wong-Staal, F.; Gallo, R.C. Science **1985, 227**, 177-182.

(3) Ho, D.D. Rota, T.R., Schooley, R.T.; et al. N. Engl. J. Med. **1985, 313**, 1493-1497.

(4) Resnick, L.; diMarzo-Veronese, F.; Schupbach, J.; et al. N. Engl. J. Med. **1985, 313**, 1498 -1504.

(5) Navia, B.A.; Jordan, B.D.; Price, R.W.**1986, 19**, 517-524.

(6) Gartner, S.; Markovits, P.; Markovitz, D.M.; Kaplan, M.H.; Gallo, R.C.; Popovic, M. Science, **1986, 233**, 215-219.

(7) Klecker, R.W.,Jr.; Collins, J.M.; Yarchoan, R.; Thomas R.; Jenkins, J.F.; Broder, S.; Myers, C.E. Clin. Pharmacol. Ther. **1987, 41**, 407-410.

(8) Yarchoan, R; Broder, S. N. Engl. J. Med. **1987, 316**, 557-564.

(9) Terasaki, T.; Pardridge, W.M. J. Infect. Diseases **1988, 158**, 630 - 632.

(10) (a) Torrence, P.F.; Kinjo, J.-e.; Lesiak, K.; Balzarini, J. De Clerq, E. FEBS Lett. **1988, 234**, 135-140; (b) Gogu, S.R.; Aggarwal, S.K.; Rangan, S.R.S.; Agrawal, K.C. J. Biopharm. Sci.**1990**,1,1-18; Aggarwal, S.K.; Gogu, S.R.; Rangan, S.R.S.; Agrawal, K.C. J. Med. Chem. **1990, 33**, 1505-1510; (d) Brewster, M. Anderson, Bodor, N. J. Pharm. Sci. 1991, 80, 843-846.

(11) For an excellent discussion of the movement of molecules through biological barriers together with a comprehensive list of references, see Bodor, N.; Brewster, M.E. Pharmac. Ther. **1983, 19**, 337-386.

(12) Mitsuya, H.; Broder, S. Proc. Natl. Acad. Sci. USA **1985, 82**, 7096-7100.

(13) Klecker, R.W.,Jr.; Collins, J.M.; Yarchoan, R.; Thomas, R.V.; McAtee, N.; Broder, S.; Myers, C.E. J. Clin. Pharmacol. **1988, 28**, 837-842.

(14) Following the completion of this study, a series of novel prodrugs of DDC were described by Kerr, S.G.; Kalman, T.I Highly

Water-Soluble Lipophilic Prodrugs of the Anti-HIV Nucleoside Analogue 2',3'-Dideoxycytidine and Its 3'-Fluoro Derivative. J. Med. Chem. **1992**, 35, 1996-2001.
(15) Horwitz, J.P.; Chua, J.; Noel, M.: Donatti, J.T. J. Org. Chem. **1967**, 32, 817-818.
(16) Brewster, M.E.;, Venkatraghavan, V.; Shek, E.; Bodor, N. Synthetic Commun. **1987**,17, 451-455.
(17) Yarchoan, R.; Thomas, R.V.; Allain, J.-P.; et al. Lancet **1988, 1**, 76-80.
(18) Skowron, G.; Merigan, T. C. Am. J. Med. **1990, 88** (supp 88, 5B), 20S-23S.
(19) Kessel, D. Biochemistry,**1977, 16**, 3443-3449.

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