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Rapid Communication

Ester prodrugs of 2′,3′-dideoxy-2′,3′-didehydrothymidine (D4T)

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Summary

Five ester prodrugs of 2′,3′-dideoxy-2′,3′-didehydrothymidine (D4T) were synthesized. The prodrugs showed higher partition coefficients to chloroform and lower solubility in water than D4T. Although the prodrugs were chemically stable within the range pH 2–7, enzymatic hydrolysis and quantitative regeneration of D4T were observed in the presence of human plasma or porcine liver esterase.

2′,3′-Dideoxy-2′,3′-didehydrothymidine (D4T) is a potent inhibitor of the reverse transcriptase of human immunodeficiency virus (HIV) isolated from patients with acquired immunodeficiency syndrome (AIDS) (Hamamoto et al., 1987; Herdewijn et al., 1987; Lin et al., 1987). Although several 2′,3′-dideoxypyrimidine nucleoside analogues have been investigated as anti-AIDS agents, rapid metabolism (Resetar and Spector, 1989), low bioavailability (Russell and Klunk, 1989) and a number of severe side effects in AIDS patients undergoing treatment (Dournon et al., 1988; Mir and Costello, 1988) have been reported. Since either these nucleoside analogues work as metabolic antagonists, or their anti-viral effects can be time-dependent, a sufficiently inhibitory concentration should be maintained in the body in order to produce the anticipated anti-viral action and to avoid undesirable side effects, such as bone marrow toxicity, which is attributable to excessive plasma concentration of drug. In order to improve delivery of the drug and/or to overcome any side effects, five novel ester prodrugs of D4T were synthesized. Their physicochemical properties and susceptibility to chemical and enzymatic hydrolysis are reported in this communication.

D4T was synthesized from thymidine according to the procedure of Horwitz et al. (1966). Briefly, thymidine was treated with 2 equiv. of methanesulfonyl chloride to yield the 3′,5′-di-*O*-mesyl derivative. Cyclization of the dimesyl derivative via the action of the aqueous NaOH led to the product 1-(2-deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)thymine. Decyclization of the oxetane ring in the latter compound was effected by potassium *t*-butoxide in dimethyl sulfoxide, affording D4T, which was purified over silica gel column chro-

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TABLE 1

Physicochemical properties of D4T prodrugs

Compound	m.p. (°C)	Partition [a] coefficient (log P)	Solubility [b] in water (μg/ml)	M.W.
D4T	167	−1.54	13360	224
Acetyl (C2-D4T)	189	0.92	7510	266
Capryl (C8-D4T)	154	2.92	5.84	350
Stearyl (C18-D4T)	135	3.55	0.088	490
Benzoyl (Bz-D4T)	155	2.47	240	328
Pivaloyl (piv-D4T)	222	2.27	52.6	308

[a] Chloroform-water, at 25 °C.
[b] At 40 °C.

matography and identified on the basis of its melting point, NMR and MS. The 5′-hydroxy position of D4T was acylated with the corresponding acid anhydride in dry pyridine to give the following ester prodrugs of D4T: acetate (C2-D4T), caprylate (C8-D4T), stearate (C18-D4T), benzoate (Bz-D4T) and pivalate (Piv-D4T). The products were purified over silica gel column chromatography, then characterized by NMR, MS and elemental analysis.

Table I lists the physicochemical properties of the D4T esters. A marked increase in the partition coefficients (chloroform/water system at 25 °C) was achieved through modification. Although the melting points were not lowered significantly by esterification, a substantial degree of solubility in water (at 40 °C) was noted for C2-D4T and Bz-D4T. The rates of chemical hydrolysis of D4T and its esters were determined at four different pH values, at 40 °C. Buffer solutions were prepared with glycine/NaOH in the case of pH 10.0, Na_2HPO_4/NaH_2PO_4 for pH 7.0, acetic acid/sodium acetate for pH 4.0, and glycine/HCl for pH 2.0. Buffer concentration was within the range 0.02–0.03 M. All buffer solutions were adjusted to a constant ionic strength of 0.02 with NaCl. Chemical hydrolysis was initiated by addition of a 10 μl stock solution (4×10^{-3} M in ethanol) to 2 ml preheated buffer solution in a glass tube. A 10 μl portion of the reaction mixture was periodically injected into a reversed-phase HPLC column (Nucleosil RP-18, 4.7 × 300 mm), and the concentration of D4T or prodrug in each sample was determined. Reactivity to chemical hydrolysis was evaluated on the basis of the pseudo-first-order rate constants obtained from the slopes of semi-logarithmic plots of ester or D4T concentration vs time (Table 2). Despite undergoing hydrolysis to

TABLE 2

Chemical and enzymatic reactivity of D4T prodrugs

Compound	Chemical hydrolysis [a]				Enzymatic hydrolysis	
	pH 2	pH 4	pH 7	pH 10	Human [b]	Porcine [c]
D4T	1.47	0.96	1.13	1.86	– [d]	– [d]
C2-D4T	4.59	0.41	0.56	20.4	0.698 (0.084)	0.118 (0.038)
C8-D4T	2.19	0.12	4.51	16.0	511 (37)	1874 (574)
C18-D4T	2.32	0.29	2.95	22.8	0.105 (0.038)	0.029 (0.005)
Bz-D4T	0.41	0.24	0.23	14.5	15.2 (2.81)	68.8 (12.1)
Piv-D4T	0.38	0.29	0.44	16.4	0.533 (0.010)	2.35 (0.28)

[a] Rate constant ($\times 10^{-4}$ min^{-1}) at 40 °C.
[b] Rate constant ($\times 10^{-2}$ min^{-1}) at 37 °C in 100% human plasma (±S.E.), $n = 3$.
[c] Rate constant ($\times 10^{-2}$ min^{-1}) at 37 °C in the presence of porcine liver esterase (1 U/ml, ±S.E.), $n = 3$.
[d] No degradation was detected up to 4 h.

D4T at pH 10, all of the esters were stable under neutral or acidic conditions (pH 2–7); the degradation products of the esters at pH 2–7 were D4T and/or thymine. Degradation of D4T proceeded slowly at pH 2–10 and yielded thymine as a product, in line with a previous report (Kawaguchi et al., 1989). The above results show that the D4T ester prodrugs are chemically stable in the body and in conventional formulations.

The rates of enzymatic hydrolysis of the esters were measured in the presence of porcine liver esterase (Sigma no. E-3128, 1–50 U/ml) or human plasma (1–100 v/v%) at 37 °C. Hydrolysis was initiated by adding stock solution to the enzyme preparation, which had been diluted with isotonic phosphate buffer (pH 7.4), to reach an initial concentration of 4 or 8×10^{-5} M. Changes in the concentration of esters and D4T were monitored by HPLC and the pseudo-first-order rate constants were determined as described above. The enzymatic reaction did not reach saturation at higher substrate concentration (8×10^{-5} M). The values of the rate constants for porcine esterase and human plasma, normalized to the concentration of 1 U/ml and 100%, respectively, are listed in Table 2. Hydrolysis of esters was observed in all cases for both enzyme systems, with D4T being regenerated quantitatively. C8-D4T showed the highest reactivity; either decrease or increase in aliphatic acyl chain length resulted in the susceptibility to both enzyme systems being reduced. Bz-D4T follows C8-D4T in order of susceptibility. The strong susceptibility of Bz-D4T to human plasma appeared characteristic on comparison with previously reported results for various esters of benzoic acid (Nielsen and Bundgaard, 1987). Piv-D4T showed a relatively low degree of susceptibility to both enzyme systems which may be attributable to steric hindrance arising from the pivaloyl moiety.

The D4T ester prodrugs demonstrate considerable chemical stability, various physicochemical properties and susceptibility to enzyme hydrolysis. Kinetic studies are currently underway in our laboratories and will be reported in the near future.

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