Nasal Absorption of 2′,3′-Didehydro-3′-deoxythymidine (D4T) and Its Esters in Rats

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Nasal absorption of 2′,3′-didehydro-3′-deoxythymidine (D4T) and its esters (5′-acetyl D4T: C2-D4T and 5′-hemisuccinyl D4T: Suc-D4T) was investigated in rats. Bioavailability of D4T following intranasal (i.n.) administration was 98.0%, and the elimination from plasma was as rapid as that following intravenous administration of D4T. There seemed to be complete and rapid absorption of D4T from the nasal cavity. The plasma concentration–time profile of D4T following i.n. administration of C2-D4T was almost the same as that after administration of D4T itself. This suggests that C2-D4T was rapidly absorbed from the nasal cavity, and that some amount of dosing C2-D4T was hydrolyzed to D4T before entering the systemic circulation. In contrast, Suc-D4T showed slower absorption in the i.n. administration, and the plasma D4T level was maintained for a long period.

Key words 2′,3′-didehydro-3′-deoxythymidine; ester prodrug; nasal absorption; rat

2′,3′-Didehydro-3′-deoxythymidine (D4T) is a 2′,3′-dideoxynucleoside analogue which has shown potent activity against human immunodeficiency virus (HIV).[1] Since D4T works as a metabolic antagonist, and its anti-HIV effect can be time-dependent, it is important that a sufficiently inhibitory concentration be maintained in the body to produce the anticipated anti-HIV effect, and to avoid undesirable side effects such as sensory peripheral neuropathy.[2,3] HIV infection frequently causes central nervous system (CNS) disorders, and thus often results in dementia.[4,5] This makes it important that anti-HIV agents be delivered effectively into CNS.

Following oral administration, D4T showed high bioavailability (72.6% in rats), while the elimination from systemic circulation was as rapid as that after intravenous (i.v.) administration.[6] However, it can be difficult to employ frequent oral administration for HIV-afflicted human patients with dementia. Intranasal (i.n.) administration is an alternative noninvasive drug delivery route which avoids the gastrointestinal-first-pass effect.[7–9] Additionally, direct transport from nasal cavity to cerebrospinal fluid (CSF) and CNS was reported,[10–16] though not discussed in this report. Passive and safe dosing through this route is available, and this could improve the compliance of patients with dementia.

In this report, we investigated nasal absorption of D4T and its esters. 5′-Acetyl D4T (C2-D4T) and 5′-hemisuccinyl D4T (Suc-D4T) were used as the ester prodrugs of D4T, their physicochemical properties and susceptibility to enzymatic hydrolysis alters the absorption from the nasal cavity.

MATERIALS AND METHODS

Materials D4T was synthesized from thymidine according to the procedure of Horwitz *et al.*[17] The 5′-hydroxy position of D4T was acylated with acetic anhydride or succinic anhydride in dry pyridine to give the ester prodrugs of D4T: acetate (C2-D4T) and hemisuccinate (Suc-D4T). These compounds were purified over silica gel column chromatography and identified on NMR and MS. All other chemicals were reagent or HPLC grade and were obtained commercially.

Animal Protocols Male Wistar rats (body weight 230—250 g) were anesthetized with 25% (w/v) of ethyl carbamate (5 ml/kg) given by intraperitoneal injection, and the femoral artery was cannulated with polyethylene tubing (SP-31). A surgical procedure for the *in vivo* nasal absorption study was carried out as described by Hirai *et al.*[7]

Nasal Absorption of Drugs The corresponding drugs were administered to rats at a dose of 44.6 μmol/kg. The preparation of D4T was solution and the prodrugs were suspended in pH 7.4 isotonic phosphate buffer. For i.n. administration, one of the drug preparations was applied to the nasal cavity through the nostril (100 μl/rat). The nostril was closed with an adhesive agent immediately after the administration.

For i.v. administration, drugs were injected as a saline solution into the tail vein of rats which were surgically operated on and treated similarly to those used in the nasal absorption study.

Blood samples were collected at 5, 10, 15, 30, 45, 60, 120 and 180 min thereafter.

Analytical Procedures An HPLC system used to determine the presence of drugs in plasma was composed of a Shimadzu LC-10A pump, a Shimadzu SPD-10A UV detector, a Rheodyne 7125 injector and a reversed-phase column (Lichrospher RP-18e, 250 × 4 mm).

The mobile phase was a mixture of water : methanol : acetic acid at 83 : 17 : 0.2 for D4T and Suc-D4T and at 70 : 30 : 0.2 for C2-D4T. The wavelength of the detection was 265 nm. The preparation of the plasma sample for D4T and its esters was the same as reported previously.[6]

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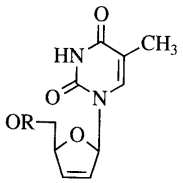
RESULTS AND DISCUSSION

Tables 1 and 2 show chemical structures and physicochemical properties, and pharmacokinetic parameters of D4T and its 5′-esterprodrugs. Figure 1a shows plasma concentration–time profile following i.v. administration of D4T at doses of 2.5, 5.0, and 10.0 mg/kg. The linear kinetics of D4T in systemic elimination was observed based on its relationship between $AUC_{0-\infty}$ and dose (Fig. 1b).

Figure 1 also shows the plasma concentration–time profile following i.n. administration of D4T. After i.n. administration, absolute bioavailability was 98.0%, maximum plasma concentration appeared at 15 min. Half life (39.6 min) calculated from the slope of the terminal phase of the profile showed no stastistically significant difference from that following i.v. administration. This observation suggests complete and rapid absorption of D4T from the nasal cavity into the systemic circulation without significant first-pass-elimination. This rapid and complete absorption of the dideoxynucleoside from the nasal cavity was consistent with our previous result with zidovudine.[16]

5′-Esterprodrugs of D4T are chemically stable and

Table 1. Chemical Structure and Physicochemical Properties of D4T and Its Esters

Chemical structure	–R	Compound	M.W.	Partition coefficient[a] (log *P*)	Solubility[b] (μg/ml)
	–H	D4T	224	−1.98	47500
	$-COCH_3$	Acetate (C2-D4T)	266	1.10	9664
	$-CO(CH_2)_2COOH$	Succinate (Suc-D4T)	324	−3.63	10600

a) Chloroform–pH 7.4 buffer, at 25 °C. *b*) In pH 7.4 buffer at 37 °C.

Table 2. Pharmacokinetic Parameters of D4T and Its Esters

Compound	Route of administration	Dose (μmol/kg)	T_{max}[a] (min)	C_{max}[b] (μM)	*AUC* (μM·h)	B.A.[e] (%)
D4T	i.v.	44.6	—	—	33.5 (3.0)[c]	—
	i.n.	44.6	15	28.5 (2.7)	32.8 (1.1)[c]	98.0
C2-D4T	i.v.	22.3	—	—	15.1 (3.1)[c]	—
	i.n.	44.6	10	28.5 (2.4)	30.3 (4.3)[c]	90.5
Suc-D4T	i.v.	44.6	—	—	20.1 (3.6)[c]	—
	i.n.	44.6	60	4.6 (0.6)	11.4 (3.3)[d]	—

a) Maximum plasma concentration time of D4T. *b*) Maximum plasma concentration of D4T. *c*) Area under the curve of plasma D4T concentration–time from time zero to infinity. *d*) Area under the curve of plasma D4T concentration–time from time zero to 180 min. *e*) Absolute bioavailability of D4T compared with *AUC* following i.v. administration of D4T. Values in parentheses indicate standard deviation of the mean value, $n=3$.

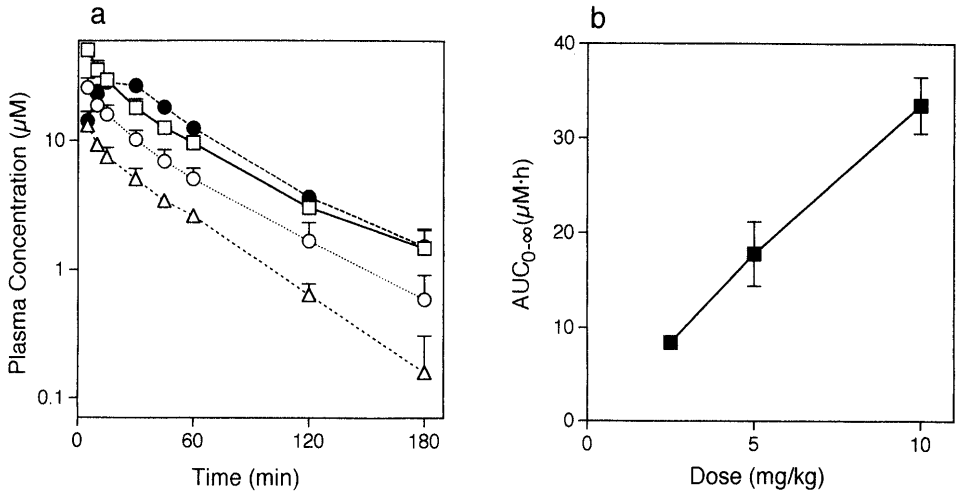


Fig. 1a. Plasma Concentration–Time Profiles following Administration of D4T

Following i.v. administration at a dose of 10 mg/kg (□), 5 mg/kg (○) and 2.5 mg/kg (△). Following i.n. administration at a dose of 10 mg/kg (●).

Fig. 1b. Relationship between *AUC* and Dose following i.v. Administration

$n=3$. Vertical bars indicate standard deviations.

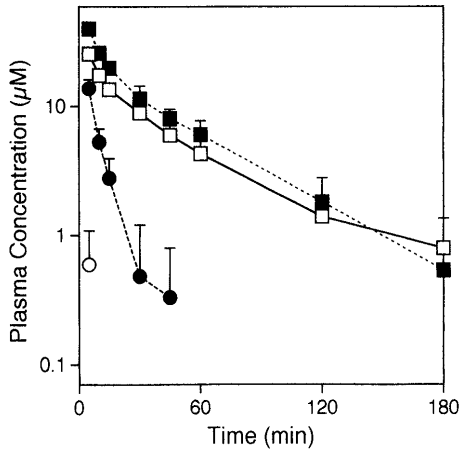


Fig. 2. Plasma Concentration–Time Profiles following i.v. Administration of C2-D4T (22.3 μmol/kg) and Suc-D4T (44.6 μmol/kg)

Detected as D4T (□) and C2-D4T (○) following administration of C2-D4T. Detected as D4T (■) and Suc-D4T (●) following administration of Suc-D4T. $n=3$. Vertical bars indicate standard deviations.

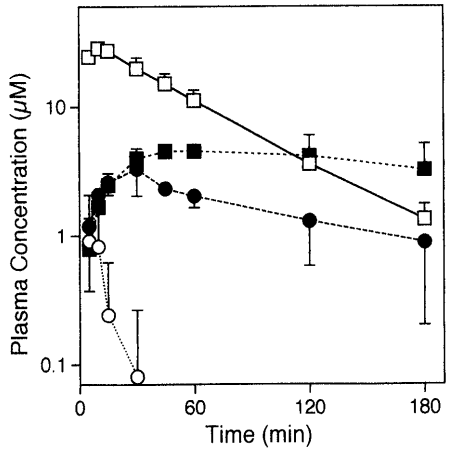


Fig. 3. Plasma Concentration–Time Profiles following i.n. Administration of C2-D4T (44.6 μmol/kg) and Suc-D4T (44.6 μmol/kg)

Detected as D4T (□) and C2-D4T (○) following administration of C2-D4T. Detected as D4T (■) and Suc-D4T (●) following administration of Suc-D4T. $n=3$. Vertical bars indicate standard deviations.

hydrolyzed enzymatically in many organs.[6,18] Plasma concentration–time profiles following i.v. administration of C2-D4T and Suc-D4T showed that the prodrug itself was detected only immediately after the administration (Fig. 2), and the plasma D4T concentration–time profiles did not greatly differ from that of D4T. This suggests rapid conversion of the prodrugs to D4T without considerable deposition to any other compartment after the i.v. administration. The relative bioavailability of D4T following i.v. administration of C2-D4T and Suc-D4T compared with that of D4T was 89.8% and 59.9%, respectively.

Figure 3 shows plasma concentration–time profiles following i.n. administration of C2-D4T. C2-D4T was detected in plasma until 30 min after the administration, whereas the elimination profile of the regenerated D4T from the plasma showed no statistically significant difference from that following i.v. administration of D4T. The plasma concentration of C2-D4T had already reached its maximum at 5 min, and decreased rapidly. C_{max} of D4T appeared at 10 min, almost the same as that after D4T administration (15 min) (Fig. 1). The bioavailability of D4T following i.n. administration of C2-D4T was 90.5% when compared with i.v. administration of D4T, suggesting that absorption of C2-D4T from the nasal cavity was rapid and complete.

Figure 3 also shows plasma concentration–time profiles following i.n. administration of Suc-D4T. The C_{max} of Suc-D4T appeared at 45 min; the Suc-D4T concentration then decreased slowly compared with that after i.v. administration of Suc-D4T. The appearance of D4T in plasma was slower than that after i.n. administration of D4T or C2-D4T, but the plasma concentration was maintained during the experimental period. The absolute bioavailability of D4T following i.n. administration of Suc-D4T cannot be calculated because the value of $AUC_{180-\infty}$ is unknown. This may be attributable to a prolonged absorption of Suc-D4T from the nasal cavity.

Huang *et al.* reported that drug molecules are mainly absorbed from nasal mucosa in their unionized form.[19] In the present study, water solubility (2.99 mg/ml in pH 3.8 buffer and 10.60 mg/ml in pH 7.4 buffer) and the chemical structure of Suc-D4T indicate that Suc-D4T molecules in the preparation for i.n. administration existed mostly in their ionized form. The partition coefficient of Suc-D4T in the chloroform–pH 7.4 buffer system also bore this out because of its low value compared with that of C2-D4T and D4T. Additionally, succinyl esters are much less labile than other alkyl esters like C2-D4T in several enzyme systems.[6] Therefore, a large part of Suc-D4T in the nasal cavity may slowly penetrate through the nasal mucosa as an intact prodrug, and this mechanism could possibly contribute to the prolonged plasma concentration following the i.n. administration.

In conclusion, our results suggest that the nasal cavity can be a suitable site for D4T administration because of its high bioavailability, while elimination from the systemic circulation was as rapid as that after intravenous administration. The i.n. administration of Suc-D4T can be useful to prolong retention of D4T in the body, and to decrease its excessive plasma concentration.

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