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2',3'-Didehydro-2',3'-dideoxythymidine (D4T), an anti-HIV agent

M. J. M. Hitchcock

Bristol-Myers Squibb Company Pharmaceutical Group,
5 Research Parkway, Wallingford, CT 06492, USA.

Introduction

D4T has been referred to by a number of names, including 2',3'-dideoxy-2',3'-didehydrothymidine, 2',3'-didehydro-3'-deoxythymidine, dideoxythymidin-2'-ene, and 1-(2,3-dideoxy- β -D-glycero-pent-2-enofuranosyl)thymine. Stavudine is the name adopted by the USAN council.

D4T is a structural analogue of the naturally occurring nucleoside thymidine. The structure of D4T is shown in Fig. 1, along with thymidine and the anti-HIV drug, 3'-azido-3'-deoxythymidine (AZT). The synthesis of D4T was first described by Horwitz *et al.* (1966) but no biological activity was reported. The discovery of activity of D4T against a mouse retrovirus (Lin *et al.*, 1987a) resurrected interest in it as a potential anti-human immunodeficiency virus (HIV) agent because the activity of other dideoxynucleosides, e.g. AZT, dideoxycytidine (ddC) and dideoxyinosine (ddI), was known. Extended biological evaluation was facilitated by the development of a scaled-up synthesis capable of producing D4T in 50-g lots (Mansuri *et al.*, 1989).

In vitro activity

Activity against HIV

A number of laboratories have detailed the activity of D4T against HIV-1 *in vitro* (Table 1). In most studies, the potency of D4T was similar to AZT in spite of significant differences in assay methodology. The exception is the data from Inoue *et al.* (1989), which shows 100-fold less activity for D4T. The anticellular activity of the two compounds is also similar (within threefold). Schreiber and Ikemoto (1988) have also reported that D4T has activity comparable to AZT against the RF strain of HIV-1 in 8166 cells. D4T was equally active against HIV-2 and HIV-1

(Balzarini *et al.*, 1989b), but in that study it was 20-fold less potent than AZT (Table 1).

Two micromolar D4T or AZT both failed to block giant cell formation caused by a range of HIV-1 isolates in chronically infected cells (Tochikura *et al.*, 1989). This was attributed to their lack of effect on the interaction of the HIV-1 envelope protein with its receptor, and is consistent with their mechanism of action (see below).

All five clinical isolates of HIV-1 from patients treated with AZT, which were less susceptible to AZT inhibition, were inhibited by D4T to a similar extent compared with the pre-therapy isolates from the same patients (Larder *et al.*, 1989, 1990). Cross-resistance of these isolates to 3'-azidodideoxyuridine and 3'-azidodideoxyguanosine implicates the role of the azido moiety rather than the thymine base.

Activity against other retroviruses

Moloney murine leukaemia virus was inhibited by D4T with an ID_{50} of 2.5 μ g/ml (Lin *et al.*, 1987a), whereas AZT was two orders of magnitude more potent in the same assay (ID_{50} of 0.02 μ g/ml). The superior potency of AZT in this system may be the result of differences in cellular pharmacology in murine cells compared to human cells; higher concentrations of AZT triphosphate are achieved in murine cells (Balzarini *et al.*, 1988).

Activity against other viruses

Recent studies have shown that D4T has no significant activity against human hepatitis B virus (Yokota *et al.*, 1991). This is in contrast to many other compounds with anti-HIV activity.

Antibacterial activity

AZT has antibacterial activity against many Gram-negative organisms (Elwell *et al.*, 1987). A direct comparison of AZT with D4T in a very limited antibacterial screen (Table 2) showed D4T was essentially inactive but confirmed the activity of AZT against enteric bacteria.

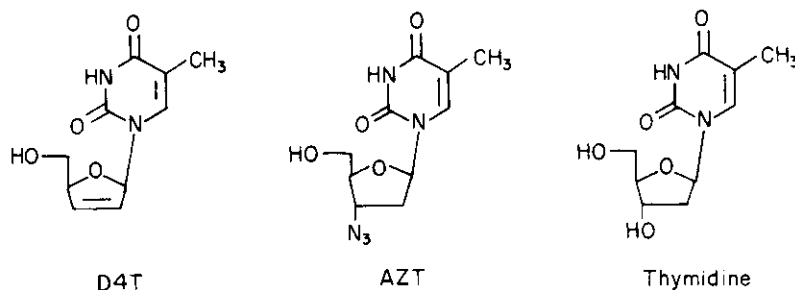


Fig. 1. Structures of D4T, AZT and thymidine.

Cytotoxicity to bone marrow progenitor cells

In order to assess *in vitro* the potential for toxicity against bone marrow progenitor cells in humans, D4T has been compared to AZT in colony-forming assays of human or murine bone marrow (Table 3). The results from different authors are somewhat at variance. In some studies, with human granulocyte/monocyte progenitors, D4T was 20–100-fold less toxic than AZT. In other studies and with other cells, the differences found were much less striking.

Mechanism of action

Metabolism of D4T to the triphosphate in cells

The metabolism of labelled D4T has been the subject of several studies using H9 (August *et al.*, 1988), CEM (Ho

and Hitchcock, 1989a), MT-4 cells (Balzarini *et al.*, 1989a) or human peripheral blood mononuclear cells (PBMC) stimulated with phytohaemagglutinin (Zhu *et al.*, 1990) or monocyte/macrophages (Perno *et al.*, 1989). In all these cells, D4T is activated to the mono-, di- and tri-phosphate. In contrast to AZT metabolism, where the major phosphorylated metabolite is the monophosphate (Furman *et al.*, 1986; Ho and Hitchcock, 1989a; Balzarini *et al.*, 1989a), D4T monophosphate does not accumulate and the ratio of mono- to di- to triphosphate is relatively constant (between 1:1:1 and 1:1:3, depending on the study). By contrast, in quiescent human peripheral blood mononuclear cells, neither D4T nor AZT are measurably phosphorylated (Ho and Hitchcock, 1989b; Zhu *et al.*, 1990), probably because thymidine kinase is not expressed under these conditions.

Table 1. Activities of D4T and AZT against HIV-1, HIV-2 and cells.

Cell type	50% inhibitory dose (ID ₅₀) (μM)					
	D4T			AZT		
	Virus	Cell	TI	Virus	Cell	TI
HIV-1						
MT-4 ^a	0.01	1.2	120	0.006	3.5	50
MT-4 ^b	0.009	119	13000	0.005		
ATH8 ^c	4.1	110	27	2.4	40–45	17–19
CEM ^d	0.15	90	600	0.1	29	20
Tall 1 ^e	0.5			0.005		
PBMC ^f	0.009–0.04	70	≥1750	0.002–0.009	200	≥2200
M/M ^g	0.05			0.1		
M/M ^h	0.3			0.2		
MT-4 ⁱ	0.05	19	380	0.003	4.8	160
HIV-2						
MT-4 ⁱ	0.09			0.004		

a. Baba *et al.* (1987).

b. Hamamoto *et al.* (1987).

c. Balzarini *et al.* (1987); Herdewijn *et al.* (1987).

d. Mansuri *et al.* (1989).

e. Inoue *et al.* (1989).

f. Lin *et al.* (1987b); Chu *et al.* (1989).

g. Monocyte/macrophage with HTLV-III_B; Perno *et al.* (1989).

h. Monocyte/macrophage with HTLV-III_{Ba-L}; Perno *et al.* (1989).

i. Balzarini *et al.* (1989b).

TI, therapeutic index (*in vitro*) = ID₅₀ for cells/ID₅₀ for virus.

Table 2. Antibacterial activities of D4T and AZT.

Organism	MIC ($\mu\text{g/ml}$)	
	D4T	AZT
<i>Staphylococcus aureus</i>	>125	125
<i>Escherichia coli</i>	>125	0.5
<i>Salmonella enteritidis</i>	125	0.5
<i>Proteus mirabilis</i>	32	8
<i>Pseudomonas aeruginosa</i>	>125	>125

MIC, minimum inhibitory concentration, determined after 18 h incubation in Mueller-Hinton broth at 37°C.

Table 3. Activities of D4T and AZT against human and murine bone marrow progenitor cells *in vitro*.

		ID ₅₀ (μM)	
		D4T	AZT
Human	CFU-GM ^{a,b}	20-100	1
	BFU-E ^{a,b}	10	5-7
	CFU-E ^c	<1	5
	CFU-G ^c	15	25
Mouse	CFU-GM ^b	11	1.5
	CFU-E (bone marrow) ^d	9.0	2.1
	CFU-E (foetal liver) ^d	2.6	1.0

a. Sommadossi *et al.* (1990); Mansuri *et al.* (1989).

b. Mansuri *et al.* (1990).

c. Inoue *et al.* (1989).

d. Gogu *et al.* (1989).

CFU, colony-forming units; BFU, burst-forming units; G, granulocyte; M, monocyte; E, erythroid.

The production of phosphorylated intracellular D4T metabolites shows a fairly good dose-response with respect to the extracellular concentration of the drug (Fig. 2). The concentration of D4T triphosphate in CEM cells increased by 100-fold for a 400-fold increase in extracellular D4T concentration (up to 20 μM). By contrast, AZT triphosphate concentration showed a poor dose-response and was increased only fourfold for a 1000-fold increase in AZT concentration. Similar effects have been described in CEM cells using higher extracellular concentrations of the two compounds (Martin *et al.*, 1991). Differences in dose-responses for the two compounds were also shown with MT-4 cells (Balzarini *et al.*, 1989a). These findings indicate that the intracellular concentrations of the active species can be more easily and predictably controlled with D4T than with AZT by modulation of the exposure, and this may lead to better predictability of clinical effects.

Since the triphosphate is believed to be the metabolite active against HIV (*vide infra*), measurement of its persistence in cells after removal of free drug from the medium is important. This creates a situation analogous to that seen after dosing animals where free drug concentrations in

plasma fall quite quickly to undetectable levels. In CEM cells, the D4T triphosphate pool decays with a half-life of about 3.5 h (Ho and Hitchcock, 1989a). Under similar conditions the half-life of AZT triphosphate was the same. Similar experiments in human PBMC also show a half-life of 3.5 h for D4T triphosphate (Zhu *et al.*, 1990). The length of the intracellular half-life of the triphosphate, coupled with the dose proportionality of triphosphate formation in cells, provided a rationale for exploring b.i.d. and t.i.d. dosing schedules in patients.

Most of the accumulated evidence points to thymidine kinase being the enzyme responsible for activation of D4T to the monophosphate. Thymidine (but not dideoxyadenosine, dideoxyinosine or deoxycytidine) added to the growth medium completely suppresses phosphorylation (Ho and Hitchcock, 1989a; Balzarini *et al.*, 1989a). Thymidine also inhibits the antiviral activity of D4T (Balzarini *et al.*, 1989a). Since thymidine is phosphorylated by thymidine kinase (TK), the result also implicates this enzyme as responsible for phosphorylation of D4T. One discrepancy is the claim that a TK-negative mutant human cell line (Raji/TK⁻) phosphorylates D4T and allows expression of anti-HIV activity (Balzarini *et al.*, 1989a). However, an alternative explanation is that the mutated TK protein retains the ability to phosphorylate D4T but not thymidine or AZT. Ho and Hitchcock (1989b) showed that D4T and AZT are phosphorylated by TK⁺ mouse cells (L1210) but not by a TK⁻ mutant mouse cell line (LMTK⁻).

Inhibition by D4T of thymidine kinase purified from CEM cells was reported (Ho and Hitchcock, 1989a). The inhibition constant (K_i) for D4T was 4600 μM compared with a Michaelis constant (K_m) of 7 μM for thymidine. This contrasts with AZT, which had a K_i of 7.6 μM under these conditions. Furman *et al.* (1986) have also presented evidence that AZT and thymidine are recognized by thymidine kinase from H9 cells in an essentially identical way, with K_m and K_i values for both being in the range of 2-3 μM . Marongiu *et al.* (1990) have also shown that D4T

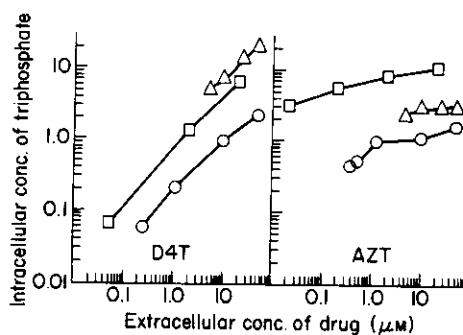


Fig. 2. Intracellular triphosphate concentrations as a function of the extracellular concentration of D4T and AZT. (□) (μM) Ho and Hitchcock (1989a); (◇) ($\text{pmol}/10^6$ cells) Balzarini *et al.* (1989a); (△) ($\text{pmol}/10^6$ cells) Martin *et al.* (1990).

has a high K_i (1370 μM) with respect to thymidine phosphorylation for thymidine kinase from H9 cells. These authors also reported its utilization as a substrate for this enzyme ($K_m = 138 \mu\text{M}$). This has been confirmed in our studies using TK from CEM cells (M. J. M. Hitchcock, unpublished data), but with a higher K_m of 1600 μM . This high value does not necessarily argue for the involvement of another enzyme, but merely indicates that the rate of phosphorylation and equilibration will not be rapid.

D4T has little effect on metabolism of exogenously labelled thymidine (Mansuri *et al.*, 1989). By contrast, AZT blocks phosphorylation of thymidine to the diphosphate. This is seen as an increase in labelled TMP and a decrease in labelled thymidine diphosphate (TDP) and thymidine triphosphate (TTP). The lack of perturbation of labelled thymidine nucleotides by D4T suggests that this compound does not modulate production of TTP, and thus synthesis of normal host DNA should not be inhibited. Furman *et al.* (1986) also described an 18-fold suppression of TTP levels by AZT in H9 cells, but subsequent studies in H9 and two other human cell lines (HL-60 and K-562) suggested that the suppression was limited and transient (Frick *et al.*, 1988).

Drug interaction experiments using labelled D4T with unlabelled AZT or *vice versa* (Ho and Hitchcock, 1989a) show that AZT inhibits phosphorylation of D4T: only 50%, 6% and 3%, respectively, of control levels of mono-, di- and tri-phosphates were found. By contrast, D4T has no effect on phosphorylation of AZT. These results, and the effects on thymidine metabolism described above, are consistent with the hypothesis that one enzyme is responsible for activation of both drugs (thymidine kinase) and

that it has a much higher affinity for AZT than D4T. The results also caution against combination of these two agents since AZT will prevent D4T from producing triphosphate and thus exerting an antiviral effect.

Inhibition by D4T triphosphate of reverse transcriptase (RT) and other DNA polymerases

The inhibitory effects of D4T and AZT triphosphates on HIV-RT is shown in Table 4. For comparison, results with other DNA polymerases are also included. In spite of some variability of the data from the different reports, it is clear that D4T-TP is a very effective inhibitor of the viral enzyme because it has a binding constant lower than that for the natural substrate, TTP. In general, its potency is similar to that of AZT-TP against RT from HIV and FIV. However, with the RT from MuLV, it is about eightfold less potent than AZT-TP. With reverse transcriptase from different sources (avian myeloblastosis virus and Rous Sarcoma virus) it has been shown that D4T-TP can terminate DNA chain elongation by being incorporated at sites normally occupied by thymidine (Dyatkina *et al.*, 1987). This mechanism has also been described for AZT-TP using *Escherichia coli* Klenow fragment (Elwell *et al.*, 1987) and using reverse transcriptase from Moloney murine leukaemia virus and HIV (St Clair *et al.*, 1987). Both the inhibition and incorporation aspects can impact on the antiviral effect by reducing the production of full length double-stranded DNA, an event in the retrovirus replication cycle needed prior to integration of the virus into the host genome.

Table 4. Kinetic constants for DNA polymerases with triphosphates of D4T, AZT and thymidine.

Polymerase	K_i (μM)		K_m (μM) TTP
	D4T-TP	AZT-TP	
HIV-RT ^a	0.032	0.007	
HIV-RT ^b	0.012	0.018	5
HIV-RT ^c	0.0083	0.0065	6.0
HIV-RT ^d		0.45	
	0.03 ^f	0.05 ^f	
FIV-RT ^e	0.0018	0.0033	3.4
R-MuLV-RT ^a	0.33	0.042	15
α^d	>200 ^f	>200 ^f	
α^e (Mg)	NI	NI	1.8
(Mn)	1.6	SI	1.7
β^d	1.0 ^f	31 ^f	
β^e (Mg)	22	SI	22
(Mn)	13	SI	17
γ^e	0.0035	0.1	0.63

a. Mansuri *et al.* (1989).

b. Mansuri *et al.* (1990).

c. North *et al.* (1990).

d. Matthes *et al.* (1987).

e. Ono *et al.* (1989).

f. Results are ID_{50} , not K_i .

NI, not inhibitory; SI, slightly inhibitory.

Cellular DNA polymerases have also been studied with triphosphates of D4T and AZT (Table 4). In one study, α -polymerase was not inhibited at very high concentrations of either compound ($>200\ \mu\text{M}$). However, Ono *et al.* (1989) showed that substitution of Mg^{2+} in the assay with Mn^{2+} allows inhibition to be seen with D4T-TP. Dyatkina *et al.* (1987), using oligonucleotide primer extension assays, have shown inhibition can be observed using $500\ \mu\text{M}$ D4T-TP and $10\ \mu\text{M}$ TTP with α -polymerase. Even under these conditions though, D4T does not appear to be irreversibly incorporated since addition of more TTP results in further extension of the DNA fragments. β -polymerase is inhibited by both D4T-TP and AZT-TP. Matthes *et al.* (1987) find D4T-TP to be 30-fold more potent than AZT-TP against the β -polymerase, and Ono *et al.* (1989) also find D4T-TP to be more inhibitory to this enzyme. It has been claimed (Dyatkina *et al.*, 1987) that with β -polymerase, D4T-TP is incorporated into DNA and terminates chain extension. There was no dose-effect, however, since no difference in termination fragments was seen at a range of D4T-TP concentrations covering two orders of magnitude. γ -Polymerase is very sensitive to inhibition by D4T-TP, and the K_i is 30-fold lower than that for AZT-TP. The development of peripheral neuropathy in patients dosed with dideoxynucleosides (see below) might be related to the interaction of their triphosphates with γ -polymerase (Starnes and Cheng, 1987) causing mitochondrial depletion (Chen and Cheng, 1989). In contrast to the result with γ -polymerase, D4T had only a small effect on incorporation of labelled precursors into DNA in isolated rat mitochondria (Simpson *et al.*, 1989). Inhibition by AZT was greater than for D4T in this system, especially when [^3H]thymidine was used.

Pharmacokinetics and metabolism *in vivo*

D4T has been shown to be stable at low pH (pH 2, Ghazzouli *et al.*, 1988; pH 1.0, Kawaguchi *et al.*, 1989). This property encouraged the exploration of oral bio-availability. In mice, oral bio-availability was 98% (Russell *et al.*, 1990) and the highest concentration in plasma was at 5 min (the first time of sampling). Thus, in mice, D4T is rapidly and completely absorbed when given by the oral route. In monkeys (Schinazi *et al.*, 1990), oral bio-availability of D4T was reported to be 42% and subcutaneous bio-availability ranged from 59% to 77%. Serum half life was between 0.8 and 1.4 h. Kaul *et al.* (1989) has also reported high plasma concentrations of D4T in rat ($200\ \mu\text{g}/\text{ml}$) and monkey ($300\ \mu\text{g}/\text{ml}$) dosed orally with 200 and 300 mg/kg D4T, respectively. Half-lives were about 0.5–0.8 h for these high doses.

Concentrations of D4T in the brain increase to $0.8\ \mu\text{g}/\text{g}$ of tissue ($\sim 3\ \mu\text{M}$) after a 25 mg/kg oral dose in mice, and then stay constant up to 60 min (Russell *et al.*, 1990). In

monkeys, the ratio of concentrations of D4T in cerebrospinal fluid to serum measured 1 h after dosing was 0.15 (Schinazi *et al.*, 1990). This ability of anti-HIV drugs to penetrate the blood–brain barrier is considered important for the treatment of acquired immunodeficiency syndrome (AIDS) dementia. !

Urinary excretion of unchanged drug over 24 h was 74%, 78% and 51% for the rat, dog, and monkey, respectively (Russell *et al.*, 1990). High-pressure liquid chromatography (HPLC) analysis of urine after administration of ^{14}C -D4T showed that essentially all the radioactivity recovered was present as D4T, and treatment of the urine with glucuronidase did not change the HPLC profile. It was concluded that D4T was not glucuronidated in any species, unlike AZT which is subject to extensive glucuronidation in monkey as well as in man (Collins and Unadkat, 1989). By contrast, Schinazi *et al.* (1990) suggested D4T glucuronide was formed to a small extent in the monkey. However, its presence was only inferred by comparisons of nucleoside concentrations before and after incubation with glucuronidase, and direct measurement of glucuronide was not performed.

Toxicology

A 30-day mouse toxicity study has been reported (Mansuri *et al.*, 1990). D4T was administered orally at doses of 100, 250, 500, and 1000 mg/kg/day and AZT was compared as a reference agent. Major haematological toxicities (anaemia, neutropaenia and lymphopaenia) were associated with AZT but not with D4T. The dose-limiting toxicity for D4T in mice was hepatotoxicity based on elevated serum alanine aminotransferase and gross or microscopic liver changes in some mice given 500 or 1000 mg/kg/day. In rats, the liver is also the organ most consistently affected by D4T. D4T at daily oral doses of 500 to 1000 mg/kg given for 1-week, 1-month or 3-months was associated with enlarged livers and hepatocellular hypertrophy in some rats (Bristol-Myers Squibb, data on file). 10-100! humo

Clinical evaluation

A pharmacokinetic study has been described in groups of five patients with AIDS or AIDS-related complex dosed orally on a q.i.d. regimen with 0.5, 1 or 2 mg/kg (S. Kaul *et al.*, Abstract S.B.455, VI International Conference on AIDS, San Francisco, 1990). T_{max} was less than 1 h and C_{max} was dose-related (Table 5). Pharmacokinetics at steady state (Day 8, 9 or 20) were consistent with those for a single dose. Bio-availability in humans was about 86% (compared with 65% for AZT) and the area under the curve (AUC) was about fourfold greater than that seen with AZT for a comparable dose (Martin *et al.*, 1990). The absence of

Table 5. Pharmacokinetics of D4T in patients with AIDS or ARC on a q.i.d. dosing regimen.

Dose (mg/kg)	Single dose			Steady state		
	0.5	1.0	2.0	0.5	1.0	2.0
C_{max} ($\mu\text{g/ml}$)	0.7	1.3	2.2	1.2	1.6	2.6
T_{max} (h)	0.5	0.8	0.7	0.5	0.8	0.6
T_{half} (h)	1.2	1.2	1.1	1.5	1.3	1.9
$AUC_{(0-8h)}$ (h/ $\mu\text{g/ml}$)	0.9	2.2	3.6	1.1	2.3	4.2

glucuronide formation may account for this increase in exposure with D4T.

A dose-escalation study has been performed in HIV-infected patients with CD4 cells $\leq 400/\text{mm}^3$ (Dunkle *et al.*, 1990; M.J. Browne *et al.*, Abstract S.B.456; K.E. Squires *et al.*, Abstract Th.A. 241, VI International Conference on AIDS, San Francisco, 1990). Doses were 2, 4, 8 and 12 mg/kg/day given orally on a t.i.d. or q.i.d. schedule. At 8 and 12 mg/kg/day, anaemia was experienced. At 2 or 4 mg/kg/day, 14 patients tolerated drug for a mean of 7 months. The consistent dose-limiting toxicity experienced was peripheral neuropathy, which had a slow onset and was reversible on discontinuation of the drug.

Beneficial responses were seen in the majority of patients. In one study, of nine patients with measurable serum p24 antigen, p24 became undetectable in eight patients within 2–5 weeks of therapy, including those patients dosed with 2 mg/kg/day (K. E. Squires *et al.*, Abstract Th.A. 241). When therapy was interrupted, serum p24 antigen levels rose rapidly to high levels, but were again suppressed by restarting therapy. In another study, with 10 patients which were positive for p24 antigen, five became negative and five showed a decline in level (M. J. Browne *et al.*, Abstract S.B. 456). Other surrogate markers for HIV disease progression (sustained increases in CD4 cells and weight gain) were also improved. Further studies designed to assess the effects of lower doses are ongoing.

Conclusion

D4T has *in vitro* activity against HIV and toxicity to cells in culture almost comparable to that of AZT. However, in some assays with human bone marrow progenitor cells (granulocyte/monocyte lineage) D4T has significantly less toxicity than AZT.

As has been shown with AZT, the nucleoside triphosphate is the active intracellular species and D4T triphosphate inhibits HIV reverse transcriptase by competing with the natural substrate, TTP. Reported binding constants (K_i) are low (about 100-fold) compared with the K_m for TTP, and similar to those measured for AZT triphosphate. Since the mechanism involves competing with the natural substrate TTP, the efficacy of inhibition *in vivo* will be likely to be dependent on the intracellular concentrations of

both of the triphosphates. (As described earlier, D4T does not suppress TTP whereas AZT may.) These triphosphates also serve as chain-terminating substrates for reverse transcriptase, causing irreversible inhibition of DNA synthesis. With mammalian polymerases (except γ -polymerase), much higher concentrations of the triphosphates are needed to inhibit DNA synthesis, and the inhibition may be reversible. Selectivity is presumably achieved from these differential effects on host and viral enzymes.

As a result of its different affinities for different enzymes, the cellular pharmacology of D4T is different from AZT. In particular, the concentration of the antiviral species, the triphosphate, has a much better dose-response to the extracellular concentration of free drug with D4T than with AZT, and D4T monophosphate does not accumulate. The ability to use the 3.5 h intracellular half-life of D4T triphosphate to support dosing frequencies of only two to three times per day is dependent on this dose-response, since the AUC of the triphosphate for each dose should be doubled by giving twice the dose at half the frequency.

In animals and humans, the oral bio-availability of D4T is consistent and relatively high (>80%), and no significant metabolites have been detected. Although the plasma half-life of the free drug is short, this may not be relevant to the dosing strategy (see above). The dose-limiting toxicity in humans is peripheral neuropathy, which was not predicted by animal toxicology. Peripheral neuropathy is also the most common dose-limiting toxicity associated with ddI or ddC therapy, and may be a class effect related to inhibition of DNA γ -polymerase. Favourable changes in the surrogate markers of HIV disease progression (p24 antigenaemia, CD4 numbers and weight gain) have been seen in a limited number of patients treated with D4T; however, the doses explored to date have produced side-effects (usually neuropathy) in a few cases after prolonged administration. Thus further clinical studies to evaluate the safety and efficacy of lower doses are currently ongoing.

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