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The transport of the anti-HIV drug, 2′,3′-didehydro-3′-deoxythymidine (D4T), across the blood-brain and blood-cerebrospinal fluid barriers

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1 The brain is a site of infection, viral replication and sanctuary for HIV-1. The treatment of HIV-1 infection therefore requires that an effective agent be delivered to the brain. 2′,3′-Didehydro-3′-deoxythymidine (D4T) is a nucleoside analogue which has been shown to have beneficial clinical effects in the treatment of HIV infection. However, although D4T has been detected in human CSF, the ability of this drug to cross both the blood-brain and blood-cerebrospinal fluid (CSF) barriers and gain entrance into the brain tissue is not known.

2 This study examined the CNS entry of D4T by means of the bilateral vascular brain perfusion technique in the anaesthetized guinea-pig.

3 The results indicated that [^{3}H]-D4T had a limited ability to cross the blood-brain barrier (BBB), which was not significantly greater than D-[^{14}C]-mannitol (a slowly penetrating marker molecule). Although D4T was found to cross the blood-CSF barrier, the presence of D4T in the CSF did not reflect levels of the drug in the brain tissue.

4 These results can be related to the measured low lipophilicity of D4T, the higher paracellular permeability characteristics of the choroid plexus (blood-CSF barrier) compared to the BBB, and the sink action nature of the CSF to the brain tissue.

5 In conclusion, these animal studies suggest that D4T may only penetrate the brain tissue to a limited extent and consideration should be given to these findings in the clinical situation.

Keywords: D4T; stavudine; blood-brain barrier; blood-CSF barrier; choroid plexus, transport; HIV; brain; CSF

Introduction

The entry of human immunodeficiency virus (HIV-1) into the central nervous system (CNS) has been shown to occur early in the course of infection and to be associated with the development of neurological disorders, such as the acquired immunodeficiency syndrome (AIDS) dementia complex (Ho *et al*., 1985; Gabuzda *et al*., 1986; Resnick *et al*., 1988). The brain has also been identified as a site of HIV-1 replication and as such may act as a reservoir for the virus, allowing the periphery to be re-infected (Black, 1985). The treatment of both the viral infection and associated CNS disorders therefore requires that an effective agent be delivered to the brain (Portegies, 1995). However, the free entry of drugs from the blood into the brain is restricted by the presence of two barriers, the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier. The BBB is found at the level of the cerebral capillary endothelium and is associated with the presence of tight junctions, metabolic enzymes, and the lack of intracellular vesicles and endothelial fenestrae (Bertler *et al*., 1966; Reese & Karnovsky, 1967; Brightman & Reese, 1969; Westergaard & Brightman, 1973; Shivers *et al*., 1984; Watson *et al*., 1991). The barrier between blood and CSF is located at the tight junctions of the choroid plexus epithelium and the dura-arachnoid boundary (Brightman & Reese, 1969; Nabeshima *et al*., 1975; Smith & Shine, 1992). The CSF bathes the exterior surfaces of the brain and fills the ventricles. Although exchange between brain and CSF is relatively unrestricted, the sink action of the CSF to the brain tissue prevents the CSF and brain interstitial fluid from being in dynamic equilibrium (Davson *et al*., 1961; Cserr *et al*., 1981; Szentesvanyi *et al*., 1984). Overall, it would justifiably appear that the blood-CSF barrier has the same functional goals as the BBB, with regard to protection and homeostasis, however the actual behaviour and ultrastructure of the choroid plexuses are quite different from that of the cerebral capillaries. The main function of the choroid plexus is to actively secrete CSF and a variety of proteins, while that of the BBB is to maintain solute gradients. In turn, both the cellular and paracellular permeability characteristics of the two barriers are quite different (Welch & Sadler, 1966; Bouldin & Krigman, 1975). Consequently the presence of a substance in the CSF does not necessarily reflect BBB permeability, but does relate to blood-CSF barrier permeability. This is of special clinical importance since the ability of a drug to cross the BBB has often been related to the presence of the drug in the lumbar CSF of patients.

The *in situ* brain perfusion technique has been developed to examine the movement of slowly moving molecules into both the brain and CSF simultaneously, and provides an opportunity to evaluate the movement of anti-HIV drugs across the blood-brain and blood-CSF barriers (Thomas & Segal, 1996; 1997a). Both 3′-azido-3′-deoxythymidine (AZT; Zidovudine) and 2′,3′-didehydro-3′-deoxythymidine (D4T; Stavudine) have been shown to have a clinical efficacy in patients with HIV-infection and are now widely used as part of combination therapies (Dudley, 1995). Clinical and basic studies have indicated that AZT can cross both the blood-brain and blood-CSF barriers and enter the brain (Portegies, 1995; Thomas & Segal, 1997a). However, although D4T has been shown to be distributed into human CSF (Dudley *et al*., 1992; Kline *et al*., 1995), the ability of D4T to enter the brain tissue has not been demonstrated. The aim of this present study was to expand on our earlier work, which characterized

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the CNS entry of thymidine and AZT (Thomas & Segal, 1996; 1997a,b) and to examine the passage of D4T across the blood-brain and blood-CSF barriers in detail. An abstract of this work has been published (Thomas & Segal, 1997c).

Methods

Bilateral carotid artery perfusion

The experimental procedures described below were carried out within the guidelines of the Animals Scientific Procedures Act 1986, U.K. Adult Dunkin-Hartley guinea-pigs (250–350 g) were anaesthetized with intramuscular injections of fentanyl-fluanisone (1 ml kg^{-1}, Hypnorm™, Janssen Pharmaceutical Ltd., Oxford, U.K. 0.315 mg ml^{-1} fentanyl citrate, 10 mg ml^{-1} fluanisone) and midazolam hydrochloride (5 mg kg^{-1}; Hypnovel™, Roche Products Ltd., Welwyn Garden City, U.K.) and then heparinized (10,000 U kg^{-1} i.p.). The common carotid arteries and the jugular veins were exposed and both carotid arteries were cannulated with silicone tubing connected to a perfusion circuit. The perfusion fluid consisted of oxygenated, twice-washed, sheep erythrocytes suspended in a saline-dextran solution (in mM: NaCl 125.1; KCl 3.9; $MgSO_4.H_2O$ 1.5; $NaHCO_3$ 25.0; KH_2PO_4 1.2; $CaCl_2.6H_2O$ 2.5; D-glucose 5; dextran (MW 70,000) 48 g l^{-1}) adjusted to a haematocrit of 20%. The perfusion fluid was thoroughly saturated with 95% O_2 and 5% CO_2 and warmed to 37°C. At the start of perfusion, the jugular veins were severed to allow drainage of the perfusion medium. The perfusion pressure and flow rate were constantly monitored and maintained at approximately 100 mmHg and 3.7 ml min^{-1}, respectively. The [^{3}H]-D4T (0.83–1.50 nM) in the absence and presence of unlabelled D4T (100 μM) or D-[^{14}C]-mannitol (0.21–0.47 μM) were then infused into the inflowing perfusion medium at a known rate (0.5 ml min^{-1}) to achieve the desired final concentration (all substrate concentrations discussed refer to final concentrations in the plasma perfusate).

Control experiments, which measured the effect of perfusion time on D-[^{14}C]-mannitol space, have shown that prolonging the perfusion period by up to 30 min does not significantly alter the permeability and therefore integrity of the BBB (Zlokovic *et al*., 1986) and blood-CSF barrier (Thomas & Segal, 1996). Further studies have also indicated that other physiological parameters, such as cerebral oxygen consumption, blood flow and water content, also remain normal during this perfusion period (Thomas & Segal, 1996). In this present study the isotope infusion period ranged from 2.5–20 min, after which a CSF sample (approximately 50 μl) was taken from the cisterna magna and perfusion terminated by decapitation. The brain was then removed, choroid plexuses excised and the brain homogenized. Perfusion fluid was collected from the carotid cannulae immediately at the end of the perfusion period. Brain tissue samples (115±0.64 mg), together with the CSF and 100 μl perfusate samples, were prepared for liquid scintillation counting. To ensure uniformity all samples were treated in a similar manner and solubilized overnight in 0.5 ml of tissue solubilizer (Soluene-350; Canberra-Packard Ltd., Pangbourne, U.K.) and, prior to isotopic counting, 3 ml of scintillation fluid (Uniscint BD, National Diagnostics, Hull, U.K.) was added. The samples were counted on a LKB-Wallac 1219 Rackbeta liquid scintillation counter which automatically corrects the c.p.m. for chemiluminescense due to the presence of a dual multi-channel analyser. Counting error was $\leqslant$3% for each sample. The corrected c.p.m. were then converted to d.p.m. by the use of internally stored quench curves (counting efficiency for ^{3}H was 63% and for ^{14}C was 97%). All samples were corrected for background (approximately 34 c.p.m. for ^{3}H and 37 c.p.m. for ^{14}C).

Capillary depletion of perfused brain

Capillary depletion analysis was used to separate the level of radiolabelled D4T that had reached the brain parenchyma from the level that was retained by the cerebral capillary endothelial cells. The brain was perfused *in situ* (20 min) and removed as described previously. Brain (0.5 g) samples were then homogenized in 3.5 mls of ice-cold physiological buffer (Bowman *et al*., 1983; Thomas & Segal, 1996). The homogenate was then diluted with ice-cold 26% dextran (MW 60,000) to achieve a final dextran concentration of 13% and the sample was re-homogenized (Triguero *et al*., 1990). An aliquot of homogenate was removed and taken for dextran-density centrifugation (5,400 × *g*; 15 min). The capillary-depleted supernatant and vasculature-enriched pellet were carefully separated. Homogenate, supernatant and pellet were solubilized, as described above, before the addition of scintillation fluid for radioactive counting.

The percent contamination of the supernatant with vessels that failed to pellet has been determined to be 4.97±0.79% (n=4), by measuring the specific activity of the vascular enzyme marker, alkaline phosphatase (Thomas & Segal, 1996). This is comparable to that originally published for the rat (Triguero *et al*., 1990). These results suggest that there is very little contamination of the supernatant by the vasculature and indicate that the supernatant mainly represents capillary-depleted brain homogenate.

Octanol/saline partition coefficients

Partition coefficients for ^{3}H-labelled D4T and D-[^{14}C]-mannitol were measured and expressed as the ratio of labelled substance found in the octanol phase to that found in the aqueous phase (Thomas & Segal, 1996). Triplicate determinations were made.

Expression of results

The R_{Tissue} % (ml g^{-1} or ml ml^{-1}) represents the amount of radioactivity in the brain (whole brain, homogenate, supernatant or pellet) or CSF (C_{Tissue}; d.p.m. g^{-1} or d.p.m. ml^{-1}) expressed as a percentage of that in the artificial plasma perfusate (C_{pl}; d.p.m. ml^{-1}). Plasma-to-CNS unidirectional transfer constants, K_{in}, were determined from the multiple-time uptake data (2.5–20 min) by means of the following equation (Patlak *et al*., 1983; Zlokovic *et al*., 1986; Thomas & Segal, 1997a):

$$\frac{C_{Tissue}\ T}{}$$

where $C_{Tissue}(T)$ and $C_{pl}(T)$ were radioactivities per unit weight of brain or CSF and plasma at time T; T being the time of perfusion in min. V_i is the initial volume of distribution of the test solute in the rapidly equilibrating space. This equation defines a straight line with a slope K_{in} (ml min^{-1} g^{-1}) and ordinate intercept V_i (ml g^{-1}).

Unidirectional transfer constants (K_{in}) represent cerebrovascular permeability surface area products (PA) and are expressed with the same units (Gjedde, 1988; Zlokovic *et al*., 1989). Backflux transport of the test solute from brain to plasma is indicated by a departure from linearity of the experimental points (Zlokovic *et al*., 1986).

Data analysis

The slopes (K_{in}) and intercept (V_i) of curves were determined by least squares linear regression analysis and are reported with their n, regression coefficients (r) and level of significance (P). Statistical analysis was performed with randomized blocks analysis of variance (ANOVA). Student's t test, modified for inequality of variances where appropriate, was used when the analysis involved the comparison of two means. Statistical significance was taken as $P<0.05$.

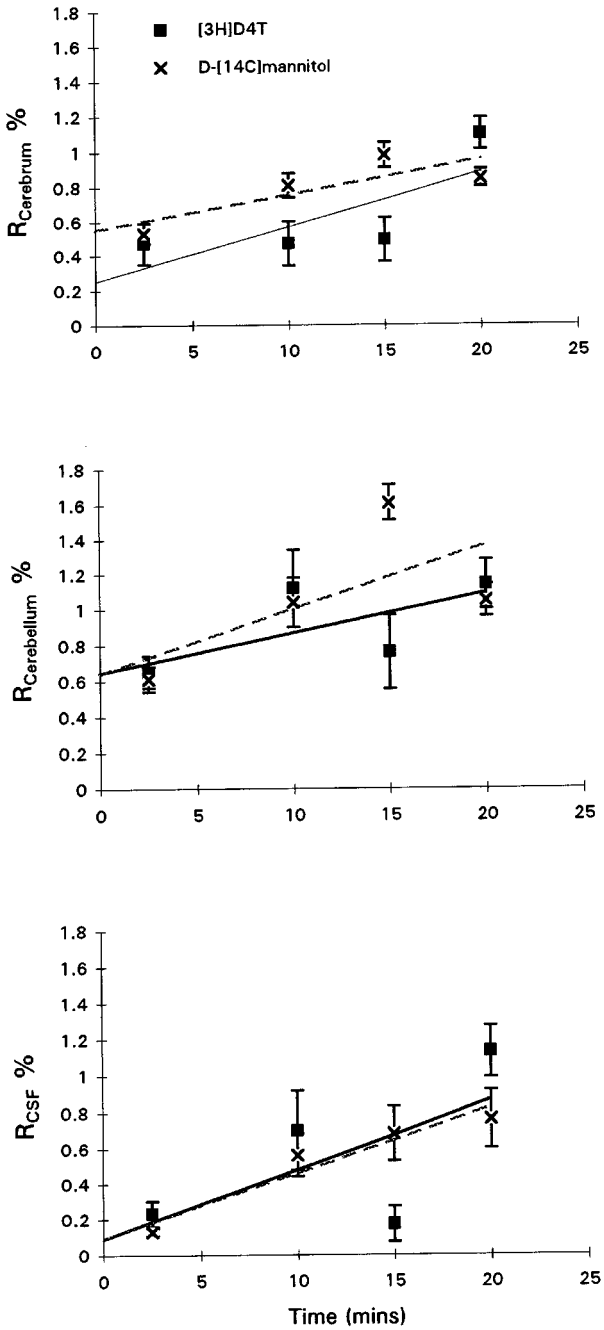

Figure 1 Brain and CSF uptake of [^{3}H]-D4T and D-[^{14}C]-mannitol plotted against time. Uptake is expressed as a percentage ratio of tissue to plasma radioactivities (R_{Tissue}%; ml 100 g^{-1}). Each point represents the mean $\pm$ s.e.mean. K_{in} and V_i values were determined as the slope and ordinate intercept of the regression lines for [^{3}H]-D4T (plain lines) and D-[^{14}C]-mannitol (dashed lines).

Materials

Unlabelled D4T was a kind gift of Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ, U.S.A.). [Methyl-^{3}H]-D4T (specific activity 18.8 Ci mmol^{-1}) and D-[^{14}C]-mannitol (specific activity 32 mCi mmol^{-1}) were purchased from Moravek Biochemicals Inc. (Brea, CA, U.S.A.) and ICN Biomedicals Ltd. (Thame, Oxfordshire, U.K.), respectively. Radiochemical purity was reported as >97% by the companies for the two compounds.

Results

The entry of [^{3}H]-D4T into the cerebrum, cerebellum and CSF was determined over perfusion periods up to 20 min and compared to the uptake of D-[^{14}C]-mannitol (Figure 1). The uptake of [^{3}H]-D4T into the cerebrum was 0.47 ± 0.12% and 1.10 ± 0.09% at 2.5 and 20 min, respectively. These values, together with those measured for the entry of [^{3}H]-D4T into the cerebellum, were found not to be significantly different to those values obtained for D-[^{14}C]-mannitol. The entry of [^{3}H]-D4T into the CSF rose from 0.23 ± 0.07% at 2.5 min to 1.13 ± 0.14% at 20 min and were not significantly different from those of D-[^{14}C]-mannitol. The results shown in Table 1 indicate that the rate of uptake (K_{in}) and initial volume of distribution (V_i) of [^{3}H]-D4T into the cerebrum, cerebellum or CSF were not significantly different from those values achieved for D-[^{14}C]-mannitol.

Capillary depletion analysis enables a more accurate quantification of the true brain uptake of labeled test drugs. The separation of the capillary endothelial cells from the brain parenchyma, identifies the fraction of the test drug which has been trapped within the endothelial cell and therefore has not actually entered the CNS. As can be seen in Table 2 all the values achieved for [^{3}H]-D4T were not significantly different to those values obtained for D-[^{14}C]-mannitol (Students t test, assuming equality of variances). Furthermore, the entry of [^{3}H]-D4T into the whole brain was found not to be significantly different to that found in the brain parenchyma or in the capillary fraction (One Way Repeated Measures ANOVA). Figure 2 illustrates the effect of 100 μM unlabelled D4T on the uptake of [^{3}H]-D4T into the cerebrum, cerebellum and CSF. As can be seen there was no significant reduction of [^{3}H]-D4T uptake into any of the tissues measured. The octanol-saline partition coefficients represent a measure of lipophilicity and were found to be 0.167 ± 0.003 and 0.002 ± 0.001 for [^{3}H]-D4T and D-[^{14}C]-mannitol, respectively.

Discussion

D4T is a pyrimidine dideoxyribonucleoside analogue which has beneficial effects on the immunological, virological and clinical markers of HIV-1 disease (Skowron, 1995). However, the presence of HIV-1 in the brain and the associated neurological disorders also requires that D4T can cross the blood-brain and blood-CSF barriers and be distributed into the brain tissue. This present study quantifies the ability of [^{3}H]-D4T to cross both these barriers.

D-[^{14}C]-mannitol is a polar sugar alcohol that only enters the CNS by simple diffusion along the restricted paracellular pathways of the cerebral capillaries and choroid plexus (Prather & Wright, 1970; Sisson & Oldendorf, 1971). Thus this marker molecule largely remains within the lumen of the cerebral capillary endothelium and can be used to measure the

Table 1 The calculated unidirectional transfer constants (K_{in}) and initial volumes of distribution (V_i) for [^{3}H]-D4T and D-[^{14}C]mannitol into the brain and CSF

	Cerebrum			*Cerebellum*			*CSF*	
Isotope	K_{in} (μl min^{-1} g^{-1})	V_i (ml 100 g^{-1})	n, r, P<	K_{in} (μl min^{-1} g^{-1})	V_i (ml 100 g^{-1})	n, r, P<	K_{in} (μl min^{-1} g^{-1})	n, r, P<
[^{3}H]-D4T	0.34±0.10	0.25±0.15	17, 0.64, 0.01	0.22±0.13	0.64±0.19	16, 0.41, NS	0.41±0.17	13, 0.59, 0.05
D-[^{14}C]-mannitol	0.20±0.05	0.55±0.07	52, 0.48, 0.001	0.35±0.10	0.64±0.13	34, 0.52, 0.005	0.36±0.09	31, 0.60, 0.001
P	NS	NS		NS	NS		NS	

Values±s.e.mean have been determined as the slope and ordinate intercept of the regression lines in Figure 1 and are reported with their *n*, regression coefficients (*r*) and level of significant (*P*). Students *t* test was used for the comparison of the K_{in} and V_i values for [^{3}H]-D4T and D-[^{14}C]-mannitol. NS is not significant.

Table 2 Distribution of [^{3}H]-D4T and D-[^{14}C]-mannitol (baseline) between the brain parenchyma and capillary endothelial cells

	R_{Tissue} %± *s.e.mean* (ml 100 g^{-1})		
Cerebrum	*[^{3}H]-D4T* ($n=5$)	*D-[^{14}C]-mannitol* ($n=4$)	P
Whole brain	0.85±0.14	0.86±0.28	NS
Brain parenchyma	0.77±0.12	0.55±0.13	NS
Capillary fraction	0.42±0.21	0.72±0.31	NS

Students *t*-test was used to compare [^{3}H]-D4T and D-[^{14}C]-mannitol values. NS is not significant. *n* is the number of experiments.

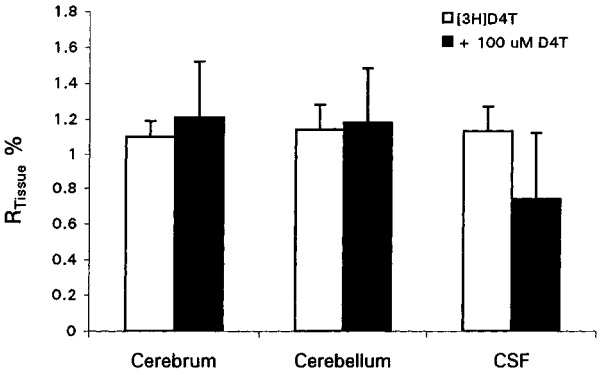

Figure 2 The effect of 100 μM unlabelled D4T on the uptake of [^{3}H]-D4T. Uptake is expressed as the percentage ratio of tissue to plasma activities (R_{Tissue}%; ml 100 g^{-1}). Values are the mean± s.e.mean for 3–6 animals.

plasma volume of the brain and the paracellular permeability of the choroid plexus. Another advantage of this internal standard is that it is of similar size (MW 182.2) to D4T (MW 224.2) and is slowly, if ever, broken down by mammalian tissue (Sisson & Oldendorf, 1971). Figure 1 and Table 1 show that there is a linear relationship between time and the uptake of D-[^{14}C]-mannitol into the brain tissue. This suggests that D-[^{14}C]-mannitol can enter the guinea-pig CNS through the paracellular pathways of the cerebral capillaries. Figure 1 also shows that the actual entry of [^{3}H]-D4T into the brain was not significantly different to that observed for D-[^{14}C]-mannitol. However, although there was a significant relationship between time and the uptake of [^{3}H]-D4T into the cerebrum, no statistically significant relationship was found between time and the uptake of [^{3}H]-D4T into the cerebellum (Table 1). This suggests that although [^{3}H]-D4T, like D-[^{14}C]-mannitol, has a very limited ability to cross the BBB, it is uncertain whether D4T can enter the brain tissue *via* the paracellular pathways of the capillaries.

Capillary depletion analysis quantifies how much radioactivity has entered the capillary endothelium (capillary fraction) and therefore determines how much radiolabelled drug has crossed these vessels at the site of the BBB and actually entered the neurones and glia (brain parenchyma). As can be seen in Table 2 all the values achieved for [^{3}H]-D4T were not significantly different to those baseline values obtained for D-[^{14}C]-mannitol. This also suggests that [^{3}H]-D4T has a very restricted transport across the BBB.

The unidirectional transfer constant (K_{in}) for the entry of [^{3}H]-D4T into the CSF was also not significantly different to that obtained for D-[^{14}C]-mannitol. This would indicate that [^{3}H]-D4T can cross the blood-CSF barrier and enter the CSF by diffusion probably *via* the paracellular route. D4T has previously been shown to be distributed into human CSF, with a CSF-to-plasma ratio of 16 to 97% having been observed (Dudley *et al*., 1992; Kline *et al*., 1995).

If we assume that the guinea-pig has a similar cerebrovascular surface area to that of the rat, then the surface area of the cerebral capillaries is approximately 155 cm^2 and that of the basolateral surface of the choroid plexus is 25 cm^2 (Keep & Jones, 1990). Thus the similar K_{in} values shown in Table 1 for the movement of [^{3}H]-D4T into the cerebellum and CSF confirm that the BBB and the blood-CSF barrier have differing permeability characteristics (Bouldin & Krigman, 1975). It has been suggested that the brain endothelium contains a low number of neutral or weakly charged, but highly permeable, hydrophilic pores (Crone, 1984). Fenstermacher & Johnson (1966) measured the effect of molecular size on the reflection coefficient of the BBB and computed a pore radius of approximately 0.8 nM. In contrast, the presence of channels 10 nM in diameter have been indicated in the choroidal epithelium (Welch & Sadler, 1966). Electrical resistance is a measure of both cellular and paracellular ionic permeability and both the cerebral endothelium and choroidal epithelium have higher electrical resistances than peripheral endothelial capillaries (approximately 1 to 2 Ω cm^2). However, the choroidal epithelium (26 Ω cm^2) has a much lower resistance than the cerebral endothelium (1462 Ω cm^2) (Zeuthen & Wright, 1981; Butt *et al*., 1990).

This present study also indicates that the entry of a drug into the CSF does not necessarily reflect levels of drug in the brain tissue. This is partly the result of the very slow turnover of brain extracellular fluid, which drains into the CSF (Cserr *et al*., 1981; Szentesvanyi *et al*., 1984), but is also related to the rate of bulk flow of the CSF out of the ventricles (Collins & Dedrick, 1983). Thus the CSF acts as a sink to the brain rather than the brain acting as a sink to the CSF (Davson *et al*., 1961).

The peak serum concentration of D4T after an oral dose (0.67–4.0 mg kg^{-1}) has been shown to be 4.4–18.5 μM (Dudley, 1995). Figure 2 shows that an excess of unlabelled

D4T (100 μM) has no significant effect on the uptake of [^{3}H]-D4T (0.83 nM) into the cerebrum, cerebellum and CSF, which would suggest that a saturable uptake system is not involved in the entry of [^{3}H]-D4T into the CNS. Therefore there is a linear relationship between concentration and the rate of transfer of D4T into the CNS. Thus the R_{Tissue} % values and the unidirectional transfer constants (K_{in}) reported in Figure 1 and Table 1 are valid for D4T concentrations of 0.83 nM to 100 μM in the perfusate, a range that includes the plasma concentrations observed in humans. The passage of D4T into human lymphocyte H-9 cells has been examined and also been found to be *via* non-facilitated diffusion (August *et al*., 1991). Furthermore, studies in an *ex vivo* model of human maternal-fetal placental transfer showed that D4T distribution was rapid and appeared to be through simple diffusion (Bawdon *et al*., 1994). D4T transfer across the Macaque placenta was also found to be passive (Odinecs *et al*., 1996)

We have previously examined the CNS entry of [^{3}H]-thymidine and [^{3}H]-AZT (Thomas & Segal, 1996; 1997a,b). Thymidine is the parent compound of both AZT and D4T and it was found to have a significantly greater rate of entry into all areas of the CNS as measured by the *in situ* brain perfusion technique in anaesthetized guinea-pigs. In addition, the rate of entry of [^{3}H]-AZT was found to be significantly greater (Students *t* test) than that for [^{3}H]-D4T into both the cerebrum ($P<0.005$) and the cerebellum ($P<0.01$) by a factor of approximately 2.7. However, the rate of entry of [^{3}H]-AZT into the CSF, as determined by multiple-time point analysis, was 0.77 ± 0.16 μl min^{-1} g^{-1} and was found not to be significantly different than that obtained for [^{3}H]-D4T. This agrees with data from a clinical trial in children (Kline *et al*., 1995) and laboratory based studies in adult rhesus monkeys and adult and infant macaques (Schinazi *et al*., 1990; Keller *et al*., 1995), which have shown that D4T and AZT enter the CSF to a similar extent.

Previously, we have shown that thymidine crosses the blood-brain and blood-CSF barriers using a saturable transport mechanism (Thomas & Segal, 1996; 1997b). In contrast, AZT was found to only enter the brain and CSF by a diffusional process (Thomas & Segal, 1997a). D4T would appear to be similar to AZT in that it enters the CNS purely by passive diffusion (Figure 2). Both D4T and AZT are thymidine analogues with a modified 2′,3′-dideoxyribose moiety containing either a 3′azido function (i.e. AZT) or with unsaturation at the 2′,3′ position (i.e. D4T). Earlier studies have observed that modification of the 3′ position of the sugar greatly reduces nucleoside interaction with the rabbit leukocyte and murine erythrocyte nucleoside transporters (Taube & Berlin, 1972; Gati *et al*., 1984). Furthermore, previous studies have demonstrated that the active nucleoside transport system in rabbit choroid plexus will transport thymidine, but not nucleoside analogues modified in the 2′-, 3′- or 5′-position (Spector, 1982; Spector & Huntoon, 1984; Wu *et al*., 1992). Thus this present study confirms that neither D4T or AZT can use the nucleoside transport processes present at the blood-brain and blood-CSF barriers of the guinea-pig. It also suggests that the absence of an affinity for the nucleoside transporter is responsible for the lower rates of CNS entry for D4T and AZT when compared to thymidine.

The octanol-saline partition coefficient determined for [^{3}H]-D4T was 0.167 and a similar value of 0.11 has previously been reported (Schinazi *et al*., 1990). The hydrophilic control, D-[^{14}C]-mannitol, mainly partitioned in the saline phase yielding a partition coefficient of 0.002. It would appear that the greater lipophilicity of AZT (octanol-saline partition coefficient of 1.020 ± 0.023) when compared to D4T, allows it to cross the BBB to a significantly greater extent (Thomas & Segal, 1997a). However, the passage of these nucleoside analogues across the blood-CSF barrier would appear to be independent of lipophilicity and, since the uptake of D4T and AZT was found not to be saturable (Figure 2; Thomas & Segal, 1997a), further suggests that their CSF entry is *via* the paracellular route. Schinazi *et al*. (1990) also found that penetration of D4T and AZT into simian CSF was independent of lipophilicity. However, they suggested that this was due to the transport of the compounds *via* a carrier-mediated process.

The rapid clinical evaluation of new drugs active against HIV requires that pharmacological properties be carefully considered to determine optimal therapies in patients (Dudley, 1995). This present study has demonstrated that the BBB permeability to [^{3}H]-D4T is very low in the anaesthetized guinea-pig. However, it would appear that [^{3}H]-D4T can cross the blood-CSF barrier *via* the paracellular route, but this pathway does not allow significant access of the drug to the brain parenchyma. Furthermore, there is no specific transport system involved in the CNS entry of [^{3}H]-D4T. Overall, this study would indicate that D4T can only penetrate the brain tissue to a small extent. Although caution must always be extended when applying the results of animal studies to the clinical setting, the results of this present study do require clinical consideration.

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References

AUGUST, E.M., BIRKS, E.M. & PRUSOFF, W.H. (1991). 3′-deoxythymidin-2′-ene permeation of human lymphocyte H9 cells by non-facilitated diffusion. *Mol. Pharmacol.,* **39,** 246 – 249.

BAWDON, R.E., KAUL, S. & DOBHI, S. (1994). The *ex vivo* transfer of the anti-HIV nucleoside compound D4T in the human placenta. *Gynecol. Obstet. Invest.,* **38,** 1 – 4.

BERTLER, A., FALCK, B., OWMAN, C.H. & ROSENGRENN, E. (1996). The localization of monoaminergic blood-brain barrier mechanisms. *Pharm. Rev.,* **18,** 369 – 385.

BLACK, P. (1985). HTLV-111, AIDS and the brain. *N. Engl. J. Med.,* **313,** 1538 – 1540.

BOULDIN, T.W. & KRIGMAN, M.R. (1975). Differential permeability of cerebral capillary and choroid plexus to lanthanum ion. *Brain Res.,* **99,** 444 – 448.

BOWMAN, P.D., ENNIS, S.R., RAREY, K.E., BETZ, A.L. & GOLDSTEIN, G.W. (1983). Brain microvessel endothelial cells in tissue culture: A model for study of blood-brain barrier permeability, *Ann. Neurol.,* **14,** 396 – 402.

BRIGHTMAN, M.W. & REESE, T.S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell. Biol.,* **40,** 648 – 677.

BUTT, A.M. JONES, H.C. & ABBOTT, N.J. (1990). Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. *J. Physiol.,* **429,** 17 – 62.

COLLINS, J.M. & DEDRICK, R.L. (1983). Distributed model for drug delivery to CSF and brain tissue, *Am. J. Physiol.,* **245,** R303 – R310.

CRONE, C. (1984). Lack of selectivity to small ions in paracellular pathways in cerebral and muscle capillaries of the frog. *J. Physiol.,* **353,** 317–337.

CSERR, H.F., COOPER, D.N., SURI, P.K. & PATLAK, C.S. (1981). Efflux of radiolabelled polyethylene glycols and albumin from rat brain, *Am. J. Physiol.,* **240,** F319–F328.

DAVSON, H., KLEEMAN, C.R. & LEVIN, E. (1961). Blood-brain barrier and extracellular space. *J. Physiol.,* **159,** 67P–68P.

DUDLEY, M.N. (1995). Clinical pharmacokinetics of nucleoside antiretroviral agents. *J. Infect. Dis.,* **171,** S99–S112.

DUDLEY, M.N., GRAHAM, K.K., KAUL, S., GELETKO, S., DUNKLE, L., BROWNE, M. & MAYER, K. (1992). Pharmacokinetics of stavudine in patients with AIDS or AIDS related complex. *J. Infect. Dis.,* **166,** 480–485.

FENSTERMACHER, J.D. & JOHNSON, J.A. (1966). Filtration and reflection coefficients of the BBB. *Amer. J. Physiol.,* **211,** 341–346.

GABUZDA, D.H., HO, D.D., DE LA MONTE, S.M., HIRSCH, M.S., ROTA, T.R. & SOBEL, R.A. (1986). Immunohistochemical identification of HTLV-111 antigen in brains of patients with AIDS. *Ann. Neurol.,* **20,** 289–295.

GATI, W.P., MISRA, H.K., KNAUS, E.E. & WIEBE, L.I. (1984). Structural modifications at the 2′- and 3′-positions of some pyrimidine nucleosides as determinants of their interaction with the mouse erythrocyte nucleoside transporter. *Biochem. Pharmacol.,* **33,** 3325–3331.

GJEDDE, A. (1988). Exchange diffusion of large neutral amino acids between blood and brain. In *Peptide and Amino Acids Transport Mechanisms in the Central Nervous System*. ed. Rakic, L., Begley, D.J., Davson, H. & Zlokovic, B.V. pp. 209–217. London, Macmillan Press.

HO, D.D., ROTA, T.R., SCHOOLEY, R.T., KAPLAN, J.C., ALLAN, J.D., GROUPMAN, J.E., RESNICK, L., FELSENSTEIN, D., ANDREWS, C.A. & HIRSCH, M.S. (1985). Isolation of HTLV-111 from cerebrospinal fluid and neural tissues of patients with neurological syndromes related to the acquired immunodeficiency syndrome. *N. Engl. J. Med.,* **313,** 1493–1497.

KEEP, R.F. & JONES, H.C. (1990). A morphometric study on the development of the lateral ventricle choroid plexus. choroid plexus capillaries and ventricular ependyma in the rat. *Dev. Brain Res.,* **56,** 47–53.

KELLER, R.D., NOSBISCH, C. & UNADKAT, J.D. (1995). Pharmacokinetics of stavudine (2′,3′-didehydro-3′-deoxythymidine) in the neonatal macaque (Macaca nemestrina). *Antimicrob. Agents. Chemother.,* **39,** 2829–2831.

KLINE, M.W., DUNKLE, L.M., CHURCH, J.A., GOLDSMITH, J.C., HARRIS, A.T., FEDERICI, M.E., SCHULTZE, M.E., WOODS, L., LOEWEN, D.F., KAUL, S., CROSS, A., RUTKIEWICZ, V.L., ROSENBLATT, H.M., HANSON, C. & SHEARER, W.T. (1995). A phase I/II evaluation of stavudine (d4T) in children with human immunodeficiency virus infection. *Pediatrics,* **96,** 247–252.

NABESHIMA, S., REESE, T.S., LANDIS, D.M.D. & BRIGHTMAN, M.W. (1975). Junctions in the meninges and marginal glia. *J. Comp. Neurol.,* **164,** 127–170.

ODINECS, A., NOSBISCH, C., KELLER, R.D., BAUGHMAN, W.L. & UNADKAT, J.D. (1996). In vivo maternal-foetal pharmacokinetics of stavudine in pigtailed macaques (Macaca nemestrina). *Antimicrob. Agents. Chem.,* **40,** 196–202.

PATLAK, C.S., FENSTERMACHER, J.D., and BLASBERG, R.G. (1983). Graphical evaluation of blood-brain barrier transfer constants from multiple-time-uptake data. *J. Cereb. Blood Flow Metab.,* **3,** 1–7.

PORTEGIES, P. (1995). HIV-1, the brain, and combination therapy. *The Lancet,* **346,** 1244–1245.

PRATHER, J.W. & WRIGHT, E.M. (1970). Molecular and kinetic parameters of sugar transport across the frog choroid plexus. *J. Membr. Biol.,* **2,** 150–172.

REESE, T.S. & KARNOVSKY, M.J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J. Cell Biol.,* **34,** 207–217.

RESNICK, L, BERGER, J.R., SHAPSHAK, P. & TOURTELLOTTE, W.W. (1988). Early penetration of the blood-brain barrier by HIV. *Neurology,* **38,** 9–14.

SCHINAZI, R.F., BOUDINOT, F.D., DOSHI, K.J. & MCCLURE, H.M. (1990). Pharmacokinetics of 3′-fluoro-3′-deoxythymidine and 3′-deoxy-2′,3′-didehydrothymidine in Rhesus monkeys. *Antimicrob. Agents. Chemother.,* **34,** 1214–1219.

SHIVERS, R.R., BETZ, A.L. & GOLDSTEIN, G.W. (1984). Isolated rat brain capillaries possess intact, structurally complex, inter-endothelial tight junctions; freeze-fracture verification of tight junction integrity. *Brain Res.,* **324,** 313–322.

SISSON, W.B. & OLDENDORF, W.H. (1971). Brain distribution spaces of mannitol-^{3}H, inulin-^{14}C and dextran-^{14}C in the rat. *Am. J. Physiol.,* **221,** 214–217.

SKOWRON, G. (1995). Biological effects and safety of stavudine: Overview of Phase 1 and 11 clinical trials. *J. Infect. Dis.,* **171,** S113–S117.

SMITH, G.M. & SHINE, H.D. (1992). Immunofluorescent labelling of tight junctions in the rat brain and spinal cord. *Int. J. Devl. Neurosci.,* **10,** 387–392.

SPECTOR, R. (1982). Pharmacokinetics and metabolism of cytosine arabinoside in the central nervous system. *J. Pharm. Exp. Ther.,* **222,** 1–6.

SPECTOR, R. & HUNTOON, S. (1984). Specificity and sodium dependence of the active nucleoside transport system in choroid plexus. *J. Neurochem.,* **42,** 1048–1052.

SZENTESVANYI, I., PATLAK, C.S., ELLIS, R.A. & CSERR, H.F. (1984). Drainage of interstitial fluid from different regions of the rat brain. *Am. J. Physiol.,* **246,** F835–F844.

TAUBE, R.A. & BERLIN, R.D. (1972). Membrane transport of nucleosides in rabbit polymorphonuclear leukocytes. *Biochim. Biophys. Acta.,* **255,** 6–18.

THOMAS, S.A. & SEGAL, M.B. (1996). Identification of a saturable uptake system for deoxyribonucleosides at the blood-brain and blood-cerebrospinal fluid barriers. *Brain Res.,* **741,** 230–239.

THOMAS, S.A. & SEGAL, M.B. (1997a). The passage of azidodeoxythymidine into and within the central nervous system: Does it follow the parent compound, thymidine? *J. Pharm. Exp. Ther.,* **281,** 1211–1218.

THOMAS, S.A. & SEGAL, M.B. (1997b). Saturation kinetics, specificity and NBMPR sensitivity of thymidine entry into the central nervous system. *Brain Res.,* **760,** 59–67.

THOMAS, S.A. & SEGAL, M.B. (1997c). Blood-to-central nervous system entry of 2′,3′-didehydro-3′-deoxythymidine and 3′-azido, 3′-deoxythymidine (abstract). *Sixth European Conference on Clinical Aspects and Treatment of HIV-infection.,* **464,** 77.

TRIGUERO, D., BUCIAK, J. & PARDRIDGE, W.M. (1990). Capillary depletion method for quantification of blood-brain barrier transport of circulating peptides and plasma proteins. *J. Neurochem.,* **54,** 1882–1888.

WATSON, P.M., ANDERSON, J.M., VANLTALLIE, C.M. & DOCTROW, S.R. (1991). The tight-junction-specific protein ZO-1 is a component of the human and rat blood-brain barriers. *Neurosci. Lett.,* **129,** 6–10.

WELCH, K. & SADLER, K. (1966). Permeability of the choroid plexus of the rabbit to several solutes. *Am. J. Physiol.,* **210,** 652–660.

WESTERGAARD, E. & BRIGHTMAN, M.W. (1973). Transport of proteins across normal cerebral arterioles. *J. Comp. Neurol.,* **152,** 17–44.

WU, X., YUAN, G., BRETT, C.M., HUI, A.C. & GIACOMINI, K.M. (1992). Sodium-dependent nucleoside transport in choroid plexus from rabbit. *J. Biol. Chem.,* **267,** 8813–8818.

ZEUTHEN, T. & WRIGHT, E.M. (1981). Epithelial potassium transport: Tracer and electrophysiological studies in choroid plexus. *J. Membr. Biol.,* **60,** 105–128.

ZLOKOVIC, B.V., BEGLEY, D.J., DJURICIC, B.M. & MITROVIC. D.M. (1986). Measurement of solute transport across the blood-brain barrier in the perfused guinea-pig brain: method and application to N-methyl-α-aminoisobutyric acid. *J. Neurochem.,* **46,** 1444–1451.

ZLOKOVIC, B.V., MACKIC, J.B., DJURICIC, B. & DAVSON, H. (1989). Kinetic analysis of leucine-enkephalin cellular uptake at the luminal side of the blood-brain barrier of an *in situ* perfused guinea-pig brain. *J. Neurochem.,* **53,** 1333–1340.

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