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Synthesis and evaluation of 5′ alkyl ester prodrugs of zidovudine for directed lymphatic delivery

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Abstract

The butanoic, lauric and oleic acid ester prodrugs of the anti-AIDS drug zidovudine (AZT) have been synthesised and assessed for their ability to promote the transport of AZT through the intestinal lymph (a major reservoir for the human immunodeficiency virus (HIV)). The octanol/water partition co-efficient and triglyceride solubility of the AZT prodrugs increased with increasing chain length of the alkyl pro-moiety, and the observed values were consistent with that required for potential intestinal lymphatic transport after oral administration. The intestinal lymphatic transport of AZT and the ester prodrugs was assessed after intraduodenal administration as a micellar lipid solution in an anesthetised rat model. Systemic blood was also sampled in order to estimate the overall extent of absorption.

The lymphatic transport of AZT was similar when administered as either AZT alone or the lipophilic ester prodrugs, where the amount of AZT collected in fistulated mesenteric lymph was approximately 0.1–0.2% of the administered dose (15 mg/kg AZT). The extent of absorption of AZT, estimated from the area under the plasma concentration time profiles of AZT, when dosed as either parent compound or the lipophilic esters, was essentially complete. These data suggest that rapid bioconversion of the ester prodrugs to AZT in either the intestinal lumen or the enterocyte limits exploitation of this approach as a means of enhancing the selective lymphatic delivery of AZT.

Keywords: AZT; Zidovudine; Lymph; Lymphatic transport; Alkyl ester prodrugs

1. Introduction

Recent data has identified lymphoid tissue as a critical repository and conduit for the human immunodeficiency virus (HIV) during infection and the progression of AIDS (Pantaleo et al.,

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1991, 1993). The majority of lymphocytes (the target for HIV) populate lymphoid tissue, only about 2% being resident in peripheral blood (Westerman and Pabst, 1990). Of these peripheral lymphocytes, only about 1% are infected throughout most of the course of an HIV infection. Redirection of the absorption pathway of orally administered anti-HIV compounds from the portal blood to the HIV-rich intestinal lymphatics may therefore significantly enhance therapy against HIV.

After oral administration, drug molecules are absorbed across the gastrointestinal tract and generally transported via the portal blood to the systemic circulation. However, in certain specialised cases, drug molecules may become associated with the triglyceride core of intestinal chylomicrons and transported to the systemic circulation via the mesenteric and thoracic lymph (Charman and Stella, 1992; Porter and Charman, 1996a). Significant drug transport via the intestinal lymphatics is limited to extremely lipophilic compounds, the common physicochemical characteristics of which are high octanol/water partition co-efficients and appreciable solubility in triglyceride lipids (Charman and Stella, 1986).

3′-Azido-3′-deoxythymidine (AZT, zidovudine) is a powerful inhibitor of reverse transcriptase (Fischl et al., 1987) and an important drug for the treatment of AIDS. However, AZT is relatively hydrophilic and is rapidly absorbed into the portal blood. In this study we have synthesised and characterised three aliphatic ester prodrugs of AZT (butyrate (C_4) ester, laurate (C_{12}) ester and oleate (C_{18}) ester) in an attempt to enhance the lymphatic transport of AZT. Simple aliphatic ester prodrugs have previously been shown to increase the intestinal lymphatic transport of testosterone esters up to 100-fold (Noguchi et al., 1985).

The utility of aliphatic ester prodrugs to enhance the intestinal lymphatic transport of AZT (and improve its therapeutic potential) was assessed in a triple-cannulated (mesenteric lymph, jugular vein and duodenum) anesthetised rat model. The resulting transport of AZT into both the portal blood and mesenteric lymphatics after administration of AZT or the prodrug is presented and the rationale for prodrug approaches for delivery of AZT to the lymph briefly discussed.

2. Materials and methods

2.1. Materials

Thymidine, diisopropyl azodicarboxylate, triphenylphosphine, butyric anhydride (99%) and Amberlite IR-120 (H^+ form) ion exchange resin, were obtained from Aldrich (Milwaukee, WI). Anisic acid (4-methoxybenzoic acid) was obtained from Ajax (Melbourne, Australia) and lithium azide was supplied by Eastman Kodak (Rochester, NY). Peanut oil, diethylaminoethyl (DEAE) Sephadex ion exchange resin (40–120 μm) and the alkyl chlorides, lauroyl chloride and oleoyl chloride, were purchased from Sigma (St. Louis, MO). Oleic acid and *n*-octanol (Ajax), mono-olein (Henkel, Germany) and polysorbate 80 (Croda, Australia) were used as received. All other solvents were at least analytical reagent grade.

2.2. Synthetic procedures

AZT was synthesised from thymidine according to the method of Czernecki and Valéry (1991). The final product was additionally purified using silica gel flash chromatography employing a 10:90 (v/v) methanol/chloroform solvent system and then recrystallised from isopropyl alcohol (uncorr. m.p. = 119–120°C). The R_f value on silica gel was 0.23 (5:95 (v/v) methanol/chloroform). ^{1}H-NMR (D_2O/tetramethylsilane (TMS)) was recorded on a Bruker AMX-300-WB spectrometer: δ 7.58 (s, 1H, 6-H); 6.14 (t, 1H, $J = 6.4$ Hz, 1′H); 4.30 (q, 1H, $J = 6.2$ Hz, 3′H); 3.96 (q, 1H, $J = 4.5$ Hz, 4′H); 3.84–3.70 (dd, 2H, $J_{5'ab} = 8.2$ Hz, $J_{5'4'} = 4.6$ Hz, 5′H); 2.45 (t, 2H, $J = 6.5$ Hz, 2′H); 1.83 (s, 3H, 5-Me). Fast atom bombardment (FAB) mass spectral analysis (Xe–thioglycerol/glycerol/methanol) recorded on a JEOL JMS-DX300 spectrometer showed peaks at 268 ($M^+ + 1$, 61.75%) and 127 ($C_5H_6N_2O_2$, thymine ring). High-resolution mass spectrometry (MS): $C_{10}H_{13}N_5O_4$ re-

quires $M^+ + 1 = 268.10458$. Found: 268.10392. IR (KBr): $\nu = 1688$, 2084, 3176 cm^{-1} were recorded on a Hitachi 270-30 spectrometer.

3′-Azido-5′-butyrate-3′-deoxythymidine: the butyric ester of AZT was synthesised by dissolving AZT (1.5 g, 5.61×10^{-3} mol) in dry pyridine (40 ml), to which 1.2 molar equivalents of butyric anhydride (1.066 g) was added and the solution stirred at room temperature overnight. The reaction mixture was concentrated at 50°C under reduced pressure, and the crude product purified using a silica gel flash chromatography column with a 5:95 (v/v) hexane/chloroform mobile phase. The fractions containing the ester were collected, identified by thin layer chromatography (TLC) and concentrated by rotary evaporation. The product was further purified by dissolving in isopropyl alcohol (50 ml) and adding 3 g of DEAE Sephadex. The mixture was stirred for 30 min, the resin removed by filtration and the product obtained by rotary evaporation. The final yield was 1.15 g (61% of theoretical yield). Recrystallisation of the butyrate ester (and subsequently the laurate and oleate esters) was unsuccessfully attempted from a number of recrystallisation solvents (*n*-hexane, diethyl ether/*n*-hexane and cyclohexane). The analytical data, proton NMR, MS, IR and high pressure liquid chromatography (HPLC) indicated pure products and, consequently, the AZT esters were left as oils at room temperature. The R_f value of the butyrate ester was 0.46 (5:95 (v/v) methanol/chloroform was 0.46). ^{1}H-NMR ($CDCl_3$/TMS): δ 8.62 (s, 1H, N–H); 7.23 (s, 1H, 6–H); 6.12 (t, 1H, $J = 6.3$ Hz, 1′H); 4.42–4.29 (dd, 2H, $J_{5'ab} = 7.9$ Hz, $J_{5'4'} = 4.4$ Hz, 5′H); 4.19 (q, 1H, $J = 7.3$ Hz, 3′H); 4.09 (q, 1H, $J = 4.4$ Hz, 4′H); 2.52–2.34 (m, 4H, 2′ and 2″ overlapped); 1.94 (s, 3H, 5-Me); 1.73–1.65 (sextant, 2H, $J = 7.4$ Hz, 3″); 0.98 (t, 3H, $J = 7.4$ Hz, terminal-CH_3). FAB mass spectral analysis (Xe–thioglycerol/glycerol/chloroform) showed peaks at 338 ($M^+ + 1$, 37.05%) and 127 ($C_5H_6N_2O_2$, thymine ring). High-resolution MS: $C_{14}H_{19}N_5O_5$ requires $M^+ + 1 = 338.14645$. Found: 338.14615. IR (NaCl): $\nu = 1656$, 2104, 3068, 3204 cm^{-1}.

3′-Azido-5′-laurate-3′-deoxythymidine: AZT (0.8 g, 2.99×10^{-3} mol) was dissolved in a mixture of dry dichloromethane (40 ml) and dry pyridine (2 equivalents). Lauroyl chloride (0.59 g, 2.69×10^{-3} mol) was added dropwise to the reaction mixture which was stirred at room temperature overnight under nitrogen. The crude product was washed with water (2×10 ml) to remove most of the pyridine·HCl salt. Final purification was achieved using silica gel flash chromatography (mobile phase 10:90 (v/v) hexane/diethyl ether). The fractions containing the lauric ester were identified by TLC, combined and concentrated by rotary evaporation. The final yield of this product was 0.95 g (79% of theoretical yield). The R_f value was 0.85 (20:80 (v/v) hexane/ethyl acetate). ^{1}H-NMR ($CDCl_3$/TMS): δ 8.2 (s, 1H, N–H); 7.23 (s, 1H, 6-H); 6.13 (t, $J = 6.3$ Hz, 1′H); 4.42–4.28 (dd, 2H, $J_{5'ab} = 7.8$ Hz, $J_{5'4'} = 4.4$, 5′H); 4.18 (q, 1H, $J = 7.5$ Hz, 3′H); 4.09 (q, 1H, $J = 4.4$ Hz, 4′H); 2.53–2.28 (m, 4H, 2′ and 2″ overlapped); 1.94 (s, 3H, 5-Me); 1.67–1.56 (m, 2H, 3″); 1.26 (broad s, 16H, methylene envelope $-(CH_2)_8-$); 0.88 (t, 3H, $J = 6.8$ Hz, terminal-CH_3). FAB mass spectral analysis (Xe-thioglycerol/glycerol/chloroform) showed peaks at 451 ($M^+ + 1$, 24.69%) and 127 ($C_5H_6N_2O_2$, thymine ring). High resolution MS: $C_{22}H_{35}N_5O_5$ requires $M^+ + 1 = 450.27164$. Found: 450.27026. IR (NaCl): $\nu = 1690$, 2104, 2860–2932, 3196 cm^{-1}.

3′-Azido-5′-oleate-3′-deoxythymidine: oleoyl chloride (1 g, 3.32×10^{-3} mol) was added dropwise to a solution of AZT (0.977 g, 3.66×10^{-3} mol) and pyridine (2 equivalents) in dry dichloromethane (50 ml). The reaction was stirred overnight at room temperature, under nitrogen. The dichloromethane solution was washed with water (2×10 ml), to remove most of the pyridine·HCl, and the product concentrated by rotary evaporation. Purification of the compound was achieved via silica gel flash chromatography (50:50 (v/v) hexane/ethyl acetate mobile phase). The yield was 1.4 g (79% of theoretical yield). The R_f value was 0.8 (mobile phase 50:50 (v/v) ethyl acetate/hexane). ^{1}H-NMR ($CDCl_3$/TMS): δ 8.27 (s, 1H, N–H); 7.22 (s, 1H, 6-H); 6.12 (t, 1H, $J = 6.3$ Hz, 1′H); 5.37–5.32 (m, broad, 2H, 9″ and 10″); 4.40–4.29 (dd, 2H, $J_{5'ab} = 7.8$ Hz, $J_{5'4'} = 4.4$ Hz, 5′H); 4.16 (q, 1H, $J = 7.3$ Hz, 3′H); 4.10 (q, 1H, $J = 4.7$ Hz, 4′H); 2.53–2.26 (m, 4H, 2′ and 2″

overlapped); 2.04–2.00 (m, 4H, 8″ and 11″); 1.94 (s, 3H, 5-Me); 1.68–1.60 (m, 2H, 3″); 1.30–1.23 (broad s, 20H, methylene envelope); 0.88 (t, 3H, $J = 6.9$ Hz, terminal-CH_3). FAB mass spectral analysis (Xe-thioglycerol/glycerol/chloroform) showed peaks at 532 ($M^+ + 1$, 32.21%) and 127 ($C_5H_6N_2O_2$, thymine ring). High resolution MS: $C_{28}H_{45}N_5O_5$ requires $M^+ + 1 = 532.34991$. Found: 532.35010. IR (NaCl): $\nu = 1688$, 2104, 2932–3016, 3208 cm^{-1}.

2.3. Log P and solubility

The method of Mack and Bönisch (1979) was modified for determination of the partition coefficient of AZT and its esters. *N*-octanol and isotonic phosphate buffer (pH 7.4), were saturated with each other prior to use. Crude amounts of each compound were weighed into separate glass vials and an equal volume (1–2 ml) of organic and aqueous phases was added to each. Distribution of AZT or ester between the two phases was obtained by manually inverting each vial for 5 min (approximately 200 inversions). Vigorous shaking was avoided to prevent formation of an emulsion at the interface. The vials were placed in an incubator maintained at 25 ± 0.1°C. On at least three separate occasions during the subsequent 48 h period, the vials were removed for short periods from the incubator, centrifuged at $2000 \times g$ for 15 min, and known aliquots of each phase analysed by HPLC. To ensure the *n*-octanol phase was fully miscible with the mobile phase, the aliquot of *n*-octanol was first dissolved in a 2:1 (v/v) methanol/chloroform co-solvent system. Distribution equilibria were recorded when the concentrations of analyte within each of the phases from consecutive readings varied by less than 5%.

The equilibrium solubility of AZT and the esters was determined in peanut oil at 25 ± 0.1°C. AZT or its esters were sequentially added to an aliquot of peanut oil until the solubility limit was exceeded. Drug and oil were then separated by centrifugation ($2600 \times g$, 5 min) and the concentration of drug in the oil determined. The esters were oil-like in their physical appearance and inspection with a polarised light source was required to determine signs of immiscibility/insolubility. Aliquots of peanut oil were initially dissolved in a 2:1 (v/v) methanol/chloroform co-solvent system, after which an appropriate dilution into the required mobile phase was performed.

2.4. AZT and AZT ester formulations

AZT and the prodrug esters were prepared in separate lipid–micellar formulations on the day of administration and the chemical stability and physical homogeneity of each verified over the period of the study. Lipid–micellar formulations were prepared by slowly introducing lipid into a rapidly stirred 8% (w/v) solution of polysorbate 80 in water. The lipid phase consisted of a 2:1 molar mixture of oleic acid and mono-olein. The solution was then diluted to a final concentration of 4% (w/v) polysorbate 80 with water and stirred for a further 10 min period. For preparation of the AZT lipid–micellar formulation, AZT was first dissolved in the external aqueous phase (containing surfactant) before addition of lipid. For the alkyl ester formulations, the ester was dissolved in the oil mixture prior to its addition to the polysorbate solution. The formulation contained 5 mg of AZT (or molar equivalent of the relevant ester), and 75 μl of lipid in the final dose volume of 2.88 ml. This is a standard formulation composition previously employed in this laboratory in lymphatic transport studies (Porter et al., 1996).

2.5. Animal experiments

The mesenteric lymph duct, jugular vein and duodenum of male Sprague–Dawley rats weighing 280–320 g were cannulated after a 24 h fast as described previously (Porter et al., 1996; Porter and Charman, 1996b). Rats were anesthetised for the duration of the experiment (intraperitoneal pentobarbitone sodium, 50 mg/kg every 2 h). After completion of the surgical procedures, the animals were placed on a heated pad maintained at 37°C (Ratex, Australia). A continuous intraduodenal infusion of normal saline at 1.44 ml/h was then initiated via a constant rate infusion

Table 1
Mobile phases and retention volumes for AZT and three 5′ alkyl ester prodrugs of AZT

Compound	Mobile phase	Retention volume (ml)
AZT	15:85 (v/v %) acetonitrile and 0.025 M phosphate buffer (pH 3.0)	5.3
Internal standard	15:85 (v/v %) acetonitrile and 0.025 M phosphate buffer (pH 3.0)	8.0
Butyrate ester of AZT	35:65 (v/v %) acetonitrile and 0.025 M phosphate buffer (pH 3.0)	8.8
Laurate ester of AZT	80:20 (v/v %) acetonitrile and 0.5% (w/w) *o*-phosphoric acid	6.5
Oleate ester of AZT	95:5 (v/v %) acetonitrile and 0.5% (w/w) *o*-phosphoric acid	7.1

pump (Bioblock Scientific, France) to maintain hydration and intestinal lymph flow. The animals were stabilized for 3 h prior to dosing. The lipid–micellar formulations (containing 5 mg of AZT, or AZT ester equivalents) were administered by intraduodenal infusion (1.44 ml/h) over a period of 2 h in place of the rehydration solution. Over the 2 h dosing period, all groups received the same volume of solution and mass of lipid. In all experimental groups, lymph was continuously collected into cooled blood collection tubes containing 7.5 mg EDTA (5 ml Vacutainer®, Becton Dickinson, NJ) which were changed at 1, 2, 3, 4, 6, 8, 10 and 12 h after the start of drug administration. Blood samples (0.5 ml) were taken from the jugular vein at −5 min and 1, 2, 3, 4, 6, 8, 10 and 12 h after the start of drug administration. At the conclusion of the experiment, animals were sacrificed by an overdose of intravenous sodium pentobarbitone and the integrity of the lymphatic and duodenal cannulas verified. The statistical significance of potential differences in the lymphatic and portal uptake of AZT and the esters was determined using one-way analysis of variance (ANOVA).

2.6. Assay method for AZT and AZT esters

The mobile phases employed for the various HPLC analyses varied in acetonitrile content depending on the compound analysed, and these are listed in Table 1. The internal standard (IS) was *p*-hydroxyphenobarbital monohydrate (99%+, Aldrich). The HPLC system was a Beckman 116 programmable solvent module (San Ramon, CA), a Beckman variable wavelength ultraviolet detector (Model 166) operated at 266 nm, a Waters 717 autosampler (Millipore, Bedford, MA) and processing of chromatographic data employed Beckman System Gold chromatographic software (version 6.0). The chromatographic column was a 4.6 mm (i.d.) 15 cm Beckman C_{18} (5 μm particle size) analytical column fitted with a 3.2 mm (i.d.) 15 mm Newguard RP 18 precolumn (Applied Biosystems, Foster City, CA). The flow rate for all analyses was 1 ml/min.

2.6.1. Plasma assay: AZT and esters

A total of 100 μl of IS solution (6.5 mg/ml) was added to 200 μl of plasma in a 12 ml polypropylene centrifuge tube. Acetonitrile (300 μl) was then added and the sample vortexed for 1 min. Ethyl acetate (4 ml) was added and the contents gently mixed on a tumble mixer for 20 min. After centrifugation at $1000 \times g$ for 5 min, 1.75 ml of the organic layer was transferred to another 12 ml polypropylene tube and evaporated to dryness under a gentle stream of nitrogen at 40°C. The sample was reconstituted in 100 μl of mobile phase, vortexed, and 50 μl subjected to HPLC analysis.

2.6.2. Lymph assay: AZT

A total of 150 μl of IS solution (6.5 mg/ml) and 4 ml of dichloromethane was added to 75 μl of lymph and the tube vortexed for 1 min. A 3 ml aliquot of the organic phase was evaporated to dryness as above and the residue reconstituted in 100 μl of mobile phase, vortexed and 50 μl subjected to HPLC analysis.

2.6.3. Lymph assay: AZT esters

tert-Butyl methyl ether (TBME) (4 ml) was added to 75 μl of lymph and the tube vortexed for

Table 2
Partition co-efficient (*n*-octanol and isotonic phosphate buffer, pH 7.4), equilibrium solubility in peanut oil and pharmacokinetic parameters after intraduodenal administration of AZT or a 5′ alkyl ester prodrug of AZT to mesenteric lymph duct cannulated rats (mean ± S.D.)

Compound	Log *P*	Equilibrium solubility in peanut oil (mg/ml)	C_{max} (μg/ml)	T_{max} (h)	Dose normalised AUC (μg·h/ml)
AZT	−0.92	<2.1	11.0 ± 3.9	2.0 ± 0.0	1.66 ± 0.81
Butyrate ester of AZT	1.40	10.8	7.4 ± 0.4	2.0 ± 0.0	1.12 ± 0.05
Laurate ester of AZT	4.07	170.5	5.2 ± 2.9	2.2 ± 0.4	0.85 ± 0.42
Oleate ester of AZT	4.51	159.8	7.4 ± 1.9	2.4 ± 0.6	1.24 ± 0.10

1 min and then centifuged at 1000 × *g* for 5 min. A 3 ml aliquot of the organic phase was evaporated to dryness and reconstituted with 100 μl of mobile phase, vortexed and 50 μl subjected to HPLC analysis.

2.6.4. Triglyceride analysis

The concentration of triglyceride in the lymph samples (expressed as mg equivalents of $C_{18:1}$ triglyceride) was determined using a DuPont Dimension AR clinical chemical analyser (Du Pont, Wilmington, DE). Endogenous triglyceride output (2.67 mg/h) was determined by collecting lymph samples from three sham-operated rats which had received a continuous duodenal infusion of normal saline. The contribution of exogenous lipid from the lipid–micelle formulation to the triglyceride content present in lymph was calculated by subtracting the nominal endogenous lipid component from the mass of triglyceride lipid determined in each collected lymph sample.

3. Results

3.1. Assay recovery, precision and accuracy

3.1.1. Plasma assay

The extraction efficiency for AZT from plasma was 91.4–95.5% (mean $n = 3$) over the range of concentrations considered (0.2–40 μg/ml). The between-day precision assessed over 3 days was less than or equal to 1.6% (c.v.%) for all AZT concentrations except for 0.2 μg/ml (6.7%). Within-day precision was 5% at all concentrations. Accuracy was 100 ± 1% (mean ± S.D.) at all concentrations except 0.2 μg/ml, which was 93.1%. Due to the large differences in lipophilicity of each ester, it was not feasible to identify an IS for each alkyl ester and consequently the determination of ester concentrations in plasma utilised a quantitative assay in which accurate volumes were taken. The extraction efficiency of the alkyl esters ranged from 87.9 to 101.9% (0.5–10 μg/ml). Within-day precision was less than 5% for all esters (3 days). Between-day variation showed a larger variation (< 10.1% except for the lauric and oleic esters at 0.5 μg/ml, which were 24.1 and 15.5%, respectively). Accuracy ranged from 98.5 to 107.5%.

3.1.2. Lymph assay

The extraction efficiency for AZT from lymph was between 78.7 and 83.5% and accuracy ranged from 96.5% at 0.5 μg/ml to 101.0% at 2 μg/ml. The highest within-day variation for AZT from lymph was 6.5% at a concentration of 2.0 μg/ml and over the 3 days of validation, the highest between-day variation was 5.1% at 0.5 μg/ml. Efficiency of extraction for the alkyl esters was in the range 89.7–94.0%, 74.1–83.9% and 77.7–83.7% for the butyrate, laurate and oleate esters, respectively. Accuracy was 100 ± 2% except for the oleate ester at 1 μg/ml which was 96.7%. Within-day variation for the alkyl esters from lymph was highest at 5.0 μg/ml for the laurate ester (8.7%). Between-day precision was less than 5% for all esters.

3.2. Physicochemical characterisation

Experimentally determined partition co-efficients, expressed as log P values for AZT and the three alkyl esters, are given in Table 2. The relative polarity of AZT is indicated by its negative log P value. The esterification of AZT imparted greater lipophilicity which increased in approximate proportion to the size of the alkyl chain. Additionally, the solubility of the alkyl AZT prodrugs in a long chain triglyceride (peanut oil) was considerably greater than that of the parent AZT, equilibrium solubility increasing from under 2.1 mg/ml for AZT to 170 mg/ml for the laurate ester. Solubility measurements are expressed as a mean of duplicate samples. Individual measurements were within 2% of the mean.

3.3. Lymphatic transport

The extent of lymphatic transport of AZT and the AZT alkyl esters was determined after intraduodenal administration to mesenteric lymph duct-cannulated anesthetised rats. The study was conducted as a four-way parallel study with at least four rats in each experimental group (AZT, $n = 4$; butyrate ester of AZT, $n = 4$; laurate ester of AZT, $n = 6$; oleate ester of AZT, $n = 5$). The rate and extent of the lymphatic transport of AZT and/or its ester are presented as the cumulative percent of administered dose in Fig. 1. Results are expressed as the mean $\pm$ S.E. ($n \geq 4$) for the sake of graphical clarity. In order to supply a lipid source to support chylomicron synthesis, AZT and the respective lipophilic AZT esters were administered in a lipid–micellar formulation. The lipophilicity of the AZT esters allowed them to be dissolved in the dispersed phase of a lipid–micellar formulation, whereas the relatively hydrophilic AZT was dissolved in the aqueous continuous phase of the lipid–micellar formulation. The total administered dose in each case was 15 mg/kg AZT (or molar equivalent of the relevant ester) and 225 μl/kg lipid (approximately 5 mg AZT and 75 μl lipid per rat).

After intraduodenal administration of AZT in the aqueous phase of a lipid–micellar formulation, 0.16% of the dose was collected in the mesenteric lymph over the 12 h period post-dose. After intraduodenal administration of the oleate ester of AZT, a small quantity of the ester was detected in lymph (15% of the total cumulative percent of dose transported into lymph). The remaining 85% of the lymphatically transported drug was present as parent AZT. However, the absolute amount of AZT transported through the lymph was still small (0.20%). Since the quantities of oleate ester reaching the lymph were small, the data for the oleic ester dosed group was expressed as total AZT reaching the lymph, i.e. from both parent and prodrug. There were no detectable quantities of the 5′ alkyl ester in the mesenteric lymph of those rats dosed with either the butyrate or laurate ester. Statistical analysis indicates that the administered esters did not improve the lymphatic uptake of drug relative to parent AZT solution ($p > 0.05$). The concentration of AZT in the mesenteric lymph as a function of time and administered prodrug is given in Fig. 2.

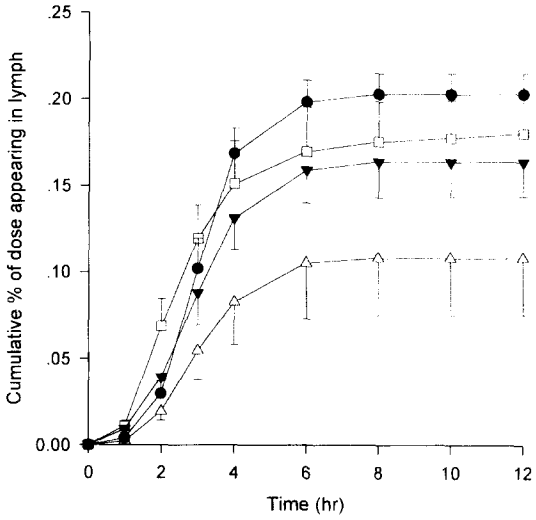

Fig. 1. Cumulative percent dose of AZT (mean $\pm$ S.E.; AZT, $n = 4$; butyrate ester of AZT, $n = 4$; laurate ester of AZT, $n = 6$; oleate ester of AZT, $n = 5$) collected in intestinal lymph as a function of time and prodrug in the triple cannulated rat. The administered dose was 5 mg AZT or molar equivalent of the relevant ester and 75 μl of lipid. AZT (▼), butyrate ester of AZT (□), laurate ester of AZT (△) and oleate ester of AZT (●).

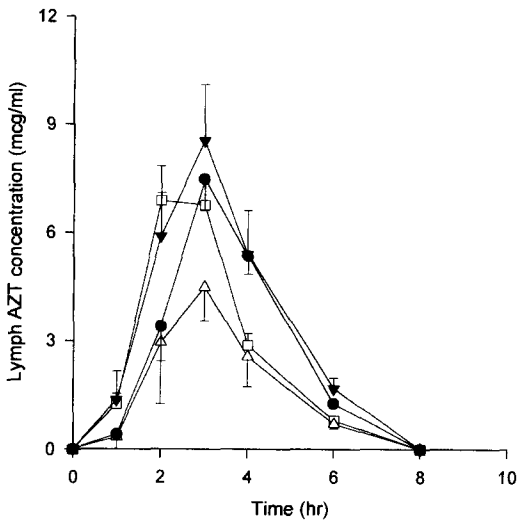

Fig. 2. Mean lymph AZT concentration–time profile after intraduodenal administration of AZT (▼), butyrate ester of AZT (□), laurate ester of AZT (△) and oleate ester of AZT (●).

3.4. AZT plasma concentration profiles

The mean plasma AZT concentration time profiles obtained after intraduodenal administration of either AZT or the AZT esters are shown in Fig. 3. Table 2 lists the pertinent pharmacokinetic parameters obtained for the four intraduodenally dosed experimental groups. The C_{max} values for AZT ranged from 5.2 ± 2.9 μg/ml after administration of the laurate ester to 11.0 ± 3.9 μg/ml after administration of the AZT lipid–micellar formulations. T_{max} values were similar across the experimental groups with mean values ranging between 2.0 and 2.4 h. No statistically significant differences were detected between the area under the curve (AUC) values of each of the experimental groups. Intravenous data obtained from five sham-operated animals (data not shown) indicate that the absorption of AZT was essentially complete.

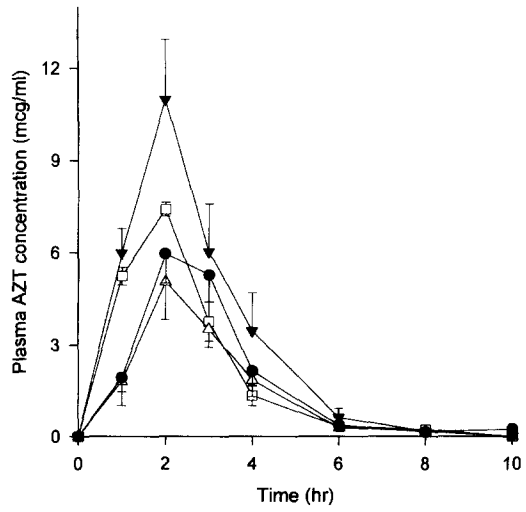

Fig. 3. Mean plasma AZT concentration–time profile after intraduodenal administration of AZT (▼), butyrate ester of AZT (□), laurate ester of AZT (△) and oleate ester of AZT (●).

3.5. Triglyceride analysis

An estimate of exogenous (formulation-derived) lipid transport into the lymph over the 12 h sampling period was determined for the experimental groups dosed with lipid–micellar formulations. The percentage of the administered lipid subsequently appearing in the intestinal mesenteric lymph was: $87.07 \pm 14.63\%$ for AZT; $53.32 \pm 11.51\%$ for the butyrate ester; $59.14 \pm 3.89\%$ for the laurate ester and $64.60 \pm 7.19\%$ for the oleate ester.

4. Discussion

The pathological progression of HIV infection and AIDS suggests that therapeutic benefit may be gained by redirecting a proportion of the dose of compounds with significant anti-HIV activity, such as AZT, to the lymphatic system and the lymphoid tissue in general. Noguchi et al. (1985) have previously demonstrated the utility of lipophilic aliphatic esters to enhance intestinal lymphatic transport, the extent of lymphatic transport of testosterone and testosterone ester being 100–1000-fold higher after administration of a palmitate or undecanoate prodrug of testosterone compared with administration of parent testosterone. In the current study, we have assessed the utility of this prodrug approach to enhance the site-specific delivery of three alkyl

ester prodrugs of AZT to the intestinal lymph in the triple cannulated laboratory rat.

The physicochemical indicators of lipophilicity (namely log P and peanut oil solubility) clearly show the enhanced lipophilic character of the prodrugs over the parent AZT (Table 2). However, unlike testosterone, after intraduodenal administration of the butyrate, laurate and oleate esters of AZT, no significant improvement was seen in the extent of lymphatic transport of AZT compared with the parent molecule (Fig. 1). The concentration of AZT in the mesenteric lymph (Fig. 2) was not significantly different to that in the systemic circulation (Fig. 3) and the quantity of AZT appearing in intestinal lymph, irrespective of the administered compound, was between 0.1 and 0.2% of the administered dose (Fig. 1). The percentage of administered lipid reaching the lymph was greater than 50% for all experimental groups, indicating that a general failure of lymphatic transport in this experimental model was not limiting lymphatic transfer. The plasma concentrations of AZT were also similar after administration of either parent drug or any of the 5′ alkyl esters. Dose-normalised AUC values for the plasma AZT concentration–time profiles of each intraduodenally administered compound (AZT or ester) were statistically indistinguishable ($p > 0.1$).

Previous investigators have demonstrated the utility of lipophilic ester prodrugs to extend the short circulatory half-life of AZT after oral administration (Kawaguchi et al., 1990, 1991). However, these studies did not address the potential contribution of lymphatic transport to the overall bioavailability of the prodrugs. The data reported here indicate that lymphatic transport does not significantly contribute to the oral bioavailability of acyl-ester prodrugs of AZT.

The in vitro susceptibility of AZT acyl-ester prodrugs to chemical hydrolysis and enzymic hydrolysis (using homogenates from mouse plasma, liver, intestine and kidney) was studied by Kawaguchi et al. (1990, 1991). The ester prodrugs were relatively stable to chemical hydrolysis at pH 4 and 7 ($< 10\%$ degradation in 100 h). Enzymic hydrolysis rates varied with the acyl chain length, short-chain and long-chain esters being relatively more stable than the medium-chain esters. The enhanced circulatory half-life described for C_2 and C_{18} ester prodrugs was ascribed to their decreased susceptibility to enzymic hydrolysis. However, the applicability of in vitro hydrolysis experiments to the study of highly lipophilic esters, where prodrug must be spiked into the enzyme reaction mixture in ethanolic solution (possibly followed by precipitation/phase separation of the lipophilic prodrug), are difficult to clearly interpret in light of the complexity of the associated physical chemistry. In vivo, lipophilic compounds are integrated into the lipid digestion cascade and distributed between emulsion, liposomal and lipid micellar dispersed phases present in the intestine (Hernell et al., 1990). The rate of hydrolysis of prodrug in these solubilised systems is likely to be significantly different to that of spiked ethanolic samples. In vitro assessment was therefore not attempted in this study. In vivo assessment of the prodrugs in a lymph cannulated model was regarded as the only reliable indicator of the extent of lymphatic transport.

The increasingly lipophilic nature of the ester prodrugs (as demonstrated by their high partition coefficient (log P) and increased capacity to dissolve in a triglyceride solvent) was expected to increase the likelihood of their being used as potential candidates for chylomicron-assisted lymphatic transport. The data reported here suggest that, unlike testosterone, the rate of hydrolysis of the AZT alkyl ester prodrugs was sufficiently high to promote hydrolysis of the prodrug before absorption into the enterocyte. The absence of steric hindrance in the comparatively non-hindered AZT molecule, relative to the steroidal nucleus of testosterone, may have permitted better approach of hydrolytic enzymes and thus explain the differences between the in vivo performance of the testosterone and AZT esters.

More complicated prodrugs such as those which integrate into lipid biochemical pathways appear to hold considerable promise for enhancing the transfer of prodrug into the lymph (Charman and Porter, 1996). Prodrugs based on mono- and diglyceride variants of drug molecules and phospholipid adducts have been shown to direct up to 30% of the administered dose into the

mesenteric lymph (Sugihara et al., 1988a,b; Sakai et al., 1993). Similar phospholipid prodrugs of AZT have been synthesised and characterised (Hostetler et al., 1990, 1994); however, the extent of lymphatic transport has yet to be elucidated. In many of the cases of successful prodrug approaches to lymphatic transport, parent drug is not found within the lymphatic compartment and is only liberated after reaching the plasma. The choice for a lymph directing prodrug moiety therefore requires a balance between stable prodrugs which circumvent the problems of pre-systemic metabolism but minimise conversion to parent drug, and more labile prodrugs which effectively liberate parent molecule but are limited by luminal hydrolysis. Stable prodrugs, where the prodrug moiety does not interfere with biological activity (and may possibly enhance activity via increased membrane transfer) appear to hold the most promise for future prodrug approaches for enhanced lymphatic transport.

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