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Pharmacokinetics and tissue distribution of long circulating liposomal formulation of 2′,3′-dideoxyinosine

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Abstract

The pharmacokinetic profiles and tissue distribution, after single bolus intravenous doses of 2′,3′-dideoxyinosine (ddI) as a solution, a conventional liposomal formulation (without polyethylene glycol) of ddI and a long circulating liposomal formulation of ddI, were determined using rat as the animal model. Total ddI concentrations displayed multicompartment features following the conventional liposome preparation and appeared one compartment after the long circulating formulation. The ddI levels after the non liposomal preparation followed a one compartment model. The conventional liposomes showed a much greater volume of distribution compared to the long circulating liposomes and subsequently a longer $t_{1/2}$. Due to the size of the conventional liposomes, these could not penetrate the fenestrated capillaries of the liver and thus had a lower accumulation in this tissue. Opsonization of these liposomes probably led to higher accumulation of the encapsulated ddI in the spleen, compared to when ddI was administered in a non liposomal form. The long circulating liposomes, on the other hand, provided a barrier to opsonin action and consequently had reduced accumulation in these tissues. The ddI levels in the pancreas were very low compared to the serum concentration after administration of the long circulating liposomes suggesting possible reduction of pancreatitis, an adverse effect of ddI. © 1997 Elsevier Science B.V.

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1. Introduction

Liposomes may be considered to be microscopic capsules that consist of one or more lipid bilayers. The pharmacokinetic profile of a drug may be completely altered by entrapping the drug in liposomes. Also, the pharmacokinetics of the liposomes themselves are different depending on the size, presence of a charge, or attachment of hydrophilic moieties on the surface of liposomes. When administered intravenously liposomes are rapidly taken up by the organs of the reticuloendothelial system (RES) and for liposomes to act

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as circulating reservoirs, they must avoid clearance by the RES (Allen, 1988). Approaches adopted for this purpose include the use of glycolipids, polysaccharides, proteins and polymers (Torchilin et al., 1980; Sunamoto and Iwamoto, 1986; Allen and Chonn, 1987; Gabizon and Papahadjopoulos, 1988; Litzinger et al., 1994). The use of polyethylene glycol-derivatized phosphatidylethanolamine (PEG-PE) in recent times has further improved the circulation time and avoidance of RES system of these liposomes.

3′-azido-3′-deoxythymidine (AZT) was one of the first anti-HIV agent used in the treatment of AIDS (Mitsuya et al., 1985). The second dideoxynucleoside administered to patients with HIV-infection was 2′,3′-dideoxycytidine (ddC) (Klecker et al., 1988). Mitsuya and Broder (1986) have reported ddC to have a similar therapeutic index as AZT but to be approximately 100 times more potent than AZT on a molar basis. A painful, sensory motor peripheral neuropathy is the dose limiting toxicity associated with ddC. 2′,3′-Dideoxyinosine (ddI), a synthetic purine nucleoside analog, has been approved by the Food and Drug Administration for the treatment of AIDS and has become a more popular drug of choice especially after the isolation of HIV strains resistant to AZT. Also, hematologic toxicity leading to anemia or leukopenia (Gill et al., 1987) and myopathy (Groopman, 1990) have been associated with long term treatment with AZT. Yarchoan et al. (1989) have identified the mechanism of action of ddI, which involves its intracellular conversion to 2′3′-dideoxyadenosine-5′-triphosphate that inhibits the action of viral reverse transcriptase, an enzyme complex that transcribes viral RNA into proviral DNA and thus blocks HIV replication. Despite the advantages of ddI over AZT in the treatment of AIDS (Kahn et al., 1992), there is some dose related toxicity associated with ddI treatment (Kamali, 1993) and include pancreatitis and peripheral neuropathy (Lipsky, 1993).

Encapsulation of ddI in liposomes could act as an ideal drug delivery system, in that it will increase efficacy, reduce toxicity, and increase the half life of ddI. It is reported in the literature that macrophages act as reservoirs for HIV (Desormeaux et al., 1994) and the encapsulation of ddI in conventional liposomes would definitely achieve the goal of passively targeting to macrophages. Other cells and tissues that can be infected by the HIV include the thymus, liver, kidney, heart, lungs, salivary glands, eyes (retina), prostate, testes, placenta and fetal tissues (Chayt et al., 1986; Barboza et al., 1992). Lasic et al. (1992) have reported that 24 h after administration of liposomes less than 1% remains in blood for conventional liposomes compared to 35% for long circulating liposomes. Encapsulation of ddI in long circulating liposomes may provide prolonged exposure of HIV infected tissues to ddI. Other than the above cells and tissues, resident macrophages and microglia in the brain have shown to be infected with the virus (Wiley et al., 1986). Siegal (1993) has demonstrated the ability of sterically stabilized liposomes to bypass the blood-brain barrier. Thus, to improve the therapeutic efficacy and at the same time reduce the toxicity of ddI, a drug delivery system with prolonged circulation time is ideal.

The purpose of this study was to determine and compare the pharmacokinetic profile and tissue distribution of ddI after single bolus IV doses of ddI as a solution, a conventional liposomal formulation of ddI, and a long circulating liposomal formulation of ddI using rat as an animal model.

2. Materials and methods

Distearoyl-L-α-phosphatidyl choline (DSPC) and polyethylene glycol-derivatized phosphatidyl ethanolamine (PEG-PE) were purchased from Avanti polar lipids (Alabaster, AL). Cholesterol (Chol) was obtained from Sigma Chemical (St. Louis, MO). [^{3}H]ddI was purchased from Moravek Biochemicals (Brea, CA). Centrifree® units were obtained from Amicon (Beverly, MA). Polycarbonate films were purchased from Nucleopore (Cambridge, MA). Solvents used were chloroform and tertiary butanol which were obtained from Fisher Scientific (Fairlawn, NJ). Scintillation cocktail (Ultima gold) and tissue solubilizer (Soluene® 350) were purchased from Packard (Meriden, CT).

2.1. Preparation of liposomes

The liposomal formulations were optimized for maximum encapsulation and best stability (Dipali et al., 1996). The formulation selected for this study was composed of DSPC and Chol in a molar ratio of 2:1 with a lipid concentration of 100 mg/ml. To prepare long circulating liposomes, 10 mol.% of PEG-PE was included in the above formulation.

Liposomes were prepared by the thin film hydration method. Weighed quantities of DSPC and Chol (and PEG-PE for long circulating liposomes) were dissolved in a mixture of tertiary butanol and chloroform (10:4) in a round bottom flask, followed by removal of the organic solvent under vacuum using a rotary evaporator. The flask was then left overnight in a vacuum desiccator to remove any solvent traces remaining in the film. A solution of ddI in phosphate buffered saline (PBS) was prepared at a concentration of 3 mg per ml of ddI and 40 μci of [^{3}H]ddI was added as a tracer. The above solution was added to the flask and the film hydrated to form multilamellar liposomes (MLVs) at 60°C which is higher than the phase transition temperature of DSPC. The MLVs were then extruded through two stacked 0.2 μm polycarbonate filters using a stainless steel extruder (Lipex Biomembranes, Vancouver, BC) to form large unilamellar liposomes (LUVs). Liposome particle size distribution and homogeneity were evaluated by large-angle dynamic light scattering (Brookhaven Instruments, Model BI-90). All particle size measurements were obtained at a temperature of 25°C, assuming a medium viscosity of 0.01P and a medium refractive index of 1.333.

The percent ddI encapsulated was determined by ultrafiltration using a Centrifree® (Amicon, Beverly, MA). There was no non-specific binding of ddI to Centrifree® filters. The LUV suspension was diluted to a lipid concentration of 5 mg/ml. Two hundred microliters of this diluted suspension was transferred to the sample reservoir of Centrifree® unit and centrifuged at 2000 rpm for 30 min, followed by two washings with 100 μl PBS and centrifugation for 10 min after each wash. The free or unencapsulated drug separated in the bottom filter cup which was mixed with 5 ml of scintillation cocktail and the radioactivity associated with ddI was measured (Beckman model LS 5000 TD).

2.2. Pharmacokinetics

Male Sprague–Dawley rats, weighing between 200–250 g, were housed in a 12-h light-dark, constant temperature environment for at least 3 days prior to the study. Food and water were freely available to the animal. The animals were randomly assigned to three treatment groups. Group I received ddI (intravenously) at a dose of 3 mg/kg, the second group received DSPC:Chol liposomes (L-DSPC) at a dose of 6 mg/kg of ddI and the third group received DSPC:Chol:PEG-PE liposomes (L-PEG) at a dose of 6 mg/kg of ddI. The liposomal formulations were administered at twice the dose of ddI alone so that the ddI levels may be comparable. The dose was administered through the jugular vein under light ether anesthesia. At the time of sampling, 10 mg/kg pentobarbital sodium in normal saline was injected intraperitoneally to anaesthetize the animal. The required amount of blood was withdrawn by means of a cardiac puncture and the animal was perfused through the heart to remove all the blood from tissues. Selected organs (liver, spleen, kidney, lung and pancreas) were then collected. The number of rats sacrificed at each time point was 3–5 and sampling times were 5, 10, 15, 30, 60, 90, 120, 180, 240 and 360 min.

Blood was allowed to coagulate and then centrifuged to collect serum. To isolate the free drug, the serum sample (200 μl) was placed in a Centrifree® tube and centrifuged at 2000 rpm for 30 min. Serum, the filtrate, and tissue samples were immediately frozen at −30°C until analysis. Concentration of ddI in serum and the filtrate were determined by HPLC. Tissue samples were treated according to a tissue solubilizer procedure (Packard, Meriden, CT) and the radioactivity measured using a liquid scintillation counter.

2.3. HPLC analysis

A reverse phase high performance liquid chromatography (HPLC) method reported by Ray and Murrill (1987) was used with minor modifications. Serum (0.5 ml) and 3 ml of acetonitrile as a protein precipitate were mixed by vortexing and centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a clean test tube and evaporated to dryness under nitrogen at 37°C. The residue was dissolved in the mobile phase (0.5 ml) and transferred to HPLC vials for analysis. The mobile phase consisted of methanol/ammonium acetate buffer (0.2 M, pH 7.0) premixed in a ratio of 12/88 v/v. A 5 μm, 100 A°, C18 column was used. A flow through beta detector (Radiomatic Flo-one Beta, Packard, Downers Grove, IL) fitted with a 0.5 ml flow cell was used to detect the drug.

2.4. Pharmacokinetic analysis

Pharmacokinetic evaluation for all the three groups was performed by model-dependent method. The serum levels for each group, were modeled by non-linear least square procedures (PC NONLIN, Version 4.2, Statistical Consultants, Lexington, KY) to one, two and three compartment models. The weighing was $1/Y^2$ and goodness of fit was based on changes in weighed sum of squares and residual plots. One-way ANOVA (Version 6.01, SAS Institute, Cary, NC) was performed to examine tissue distribution differences using SAS. Data are reported as mean $\pm$ SD except for results from pharmacokinetic modeling which was mean $\pm$ SE.

3. Results and discussion

The formulations prepared for this study consisted of 39% encapsulated and 61% free ddI in case of L-DSPC and 45% encapsulated and 55% free in case of L-PEG. Because a significant amount of the total ddI was present in the form of unencapsulated drug, the pharmacokinetic parameters and models were determined for encapsulated drug, unencapsulated drug and total (free + encapsulated) drug. The nonencapsulated drug was not separated because of lower encapsulation efficiency of ddI. If we were to remove the free drug that would have resulted into administering larger volume of liposome samples to rats which is not practical. Unfortunately, with the L-DSPC studies the total ddI levels could only be modeled since the levels of the free ddI from this formulation were below detectable levels after the first sampling period.

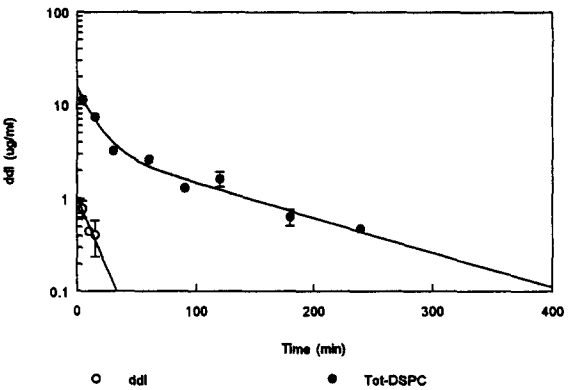

Fig. 1. Serum ddI concentration profiles after single intravenous dose of non liposomal ddI at 3 mg/kg of ddI and DSPC liposomes at 6 mg/kg of ddI.

The mean serum concentration profile of ddI after 3 mg/kg ddI IV and after 6 mg/kg ddI as L-DSPC are shown in Fig. 1. Fig. 2 represents the mean serum concentration profile of unencapsulated ddI, encapsulated ddI and total ddI after 6 mg/kg of ddI as L-PEG.

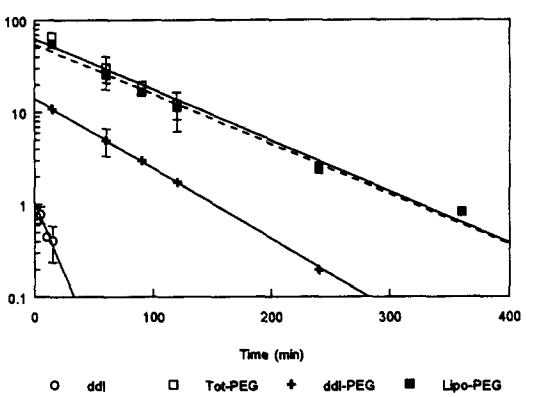

Fig. 2. Free, encapsulated and total (free + encapsulated) ddI concentration profiles after single intravenous dose of DSPC:Chol:PEG liposomes at 6 mg/kg of ddI.

As seen in Fig. 1, ddI after IV bolus follows a one compartment model which has been reported in literature (Desormeaux et al., 1994; Ray et al., 1990). Following administration of L-PEG (Fig. 2) ddI levels also followed a one compartment model for encapsulated ddI, unencapsulated ddI and total ddI. The half lives of free ddI (0.647 min) was similar to the half life of liposomal ddI (0.752 min) suggesting the slow release of ddI than the L-PEG formulation (Table 1). This observation was also similar to literature reports with other long circulating liposomes (Allen and Hansen, 1991). L-DSPC on the other hand shows a biphasic profile for total ddI which has been noted for other conventional liposomes (Allen and Hansen, 1991; Gay et al., 1993). The biphasic profile is believed to be due to fast elimination of free drug as well as the initial rapid uptake of these liposomes by the mononuclear phagocyte system (MPS). For L-DSPC, based on total ddI, its initial volume of distribution was 0.372 (0.0504) l/kg while its steady state volume of distribution was 0.861 (0.105) l/kg as calculated from total ddI.

Table 1 lists the calculated model dependent pharmacokinetic parameters based on total ddI. The L-DSPC displays a much greater Cl_T (0.62 l/h per kg) than the L-PEG (0.071 l/h per kg). The conventional liposomes (L-DSPC) had a much larger volume of distribution (0.861 l/kg) as compared to the stealth (Stealth® is a registered trade mark of Liposome Technology for liposomes containing PEG groups on liposome surface) liposomes (L-PEG, 0.074 l/kg). Despite a higher Cl_T, the L-DSPC formulation displayed a longer $t_{1/2}$ (1.24 h) for total ddI compared to L-PEG (0.726 h). This is probably due to the larger volume of distribution of L-DSPC compared to L-PEG. The biphasic decline of total ddI concentrations after L-DSPC may be due to this conventional liposome's rapid uptake into the MPS as well as elimination of the ddI. This initial phase is followed by slower uptake which may be dependent on clearance of these liposomes from the MPS. The very low volume of distribution (0.091 l/kg) of ddI in L-PEG compared to 2.75 l/kg for ddI IV and 0.86 l/kg for L-DSPC shows that the PEG liposomes have markedly reduced distribution out of the plasma compared to ddI and the L-DSPC liposomes.

The tissue levels of ddI at different time points after the three treatments are shown in Fig. 3. The levels following ddI IV have been normalized (doubled) to a dose of 6 mg/kg for comparison purposes. The normalization was considered since the pharmacokinetics of ddI is reported to be independent of dose (McGowan et al., 1990). Even though some of the tissue levels in Fig. 3 look similar among the three groups, a marked difference can be seen when comparing the tissue/serum ratios. Fig. 4 shows the tissue/serum ratios at 15, 60 and 120 min.

From Fig. 3 it is seen that the ddI levels in the liver are similar for all three treatments. However, the liver/serum ratio over 15–120 min, in the case of L-DSPC was about 0.5 and that for L-PEG was 0.1 at 15 min and gradually increases to 0.22 at 4 h. The long circulating L-PEG liposomes are not recognized by the liver macrophages and hence the lower uptake. The lower uptake of ddI in conventional liposomes is because of the size of the liposomes which was about 240 nm. It is a well documented fact that larger liposomes of the size used in this study cannot penetrate the fenestrated capillaries of the liver and thus display lower accumulation (Desormeaux et al., 1994; Roerdink et al., 1981; Rahman et al., 1982).

It is reported in the literature that liver takes up cholesterol-free more than the cholesterol-rich liposomes, whereas the spleen prefers cholesterol-rich over cholesterol-poor liposomes (Moghimi and Patel, 1988, 1989). Since the liposomes used in this study contain 50 mole% cholesterol, high accumulation in the spleen is expected. Due to opsonization, L-DSPC is taken up into the spleen to a greater degree than L-PEG. The presence of PEG provides a steric barrier to opsonins (Klibanov et al., 1991; Mori et al., 1991) as well as increases the hydrophilicity (Allen et al., 1991; Klibanov et al., 1990) at the liposome surface which reduces interaction with opsonin molecules. The ddI concentration in the spleen after administration of L-DSPC is at least twice as much compared to the levels after administration of L-PEG (Fig. 3). This difference is more apparent when we compare the ratio of spleen to serum

Table 1
Model dependent pharmacokinetic parameters of ddI following single intravenous dose of non liposomal ddI, a conventional liposomal formulation of ddI and a long circulating liposomal formulation of ddI

Formulation	Volume of distribution (l/kg)	Elimination rate constant (h^{-1})	AUC (μg h/ml)	Half-life (h)		Total body clearance (l/h per kg)
				$T_{1/2\alpha}$	$T_{1/2\beta}$	
Non-liposomal ddI	2.751 (0.635)	4.500 (1.459)	0.242 (0.039)		0.154 (0.050)	12.380 (1.970)
DSPC:Chol				0.142 (0.034)		
Total	0.861 (0.105)	0.560 (0.106)	9.678 (0.578)		1.237 (0.234)	0.620 (0.037)
DSPC:Chol:PEG-PE						
Unencapsulated	0.427 (0.035)	1.072 (0.103)	13.100 (0.830)		0.647 (0.062)	0.458 (0.029)
Encapsulated	0.091 (0.008)	0.922 (0.086)	71.900 (4.630)		0.752 (0.070)	0.084 (0.005)
Total	0.074 (0.006)	0.955 (0.078)	84.490 (4.840)		0.726 (0.060)	0.071 (0.004)

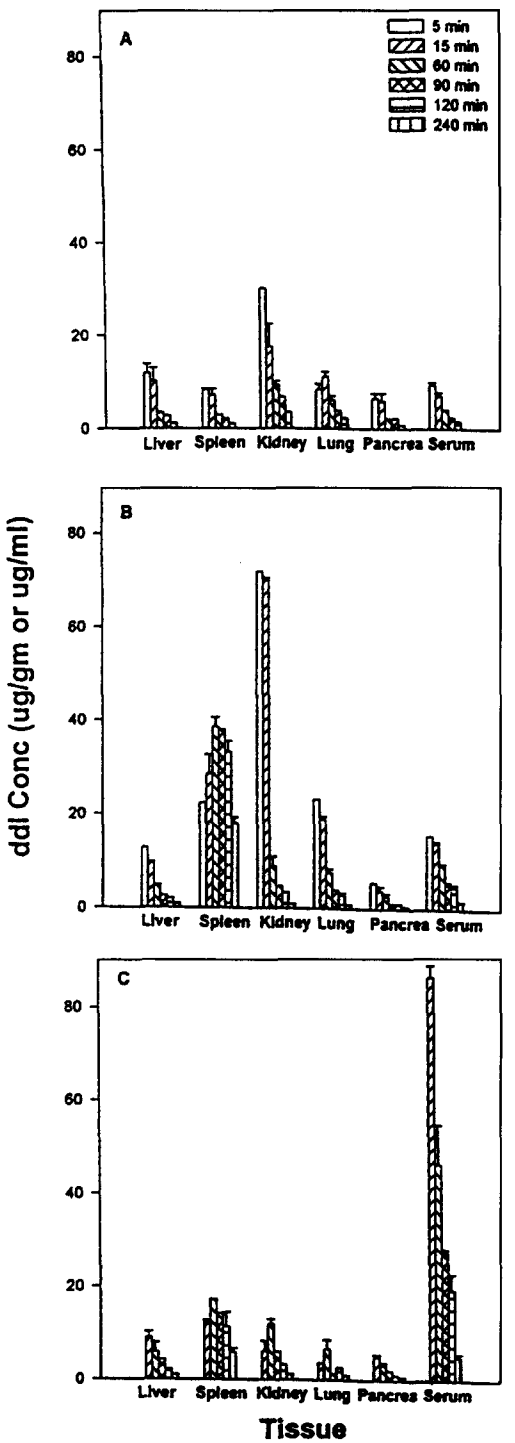

Fig. 3. Tissue and serum concentration profiles of ddI after: (A) non liposomal ddI, (B) DSPC liposomes and (C) DSPC:Chol:PEG liposomes (note: Levels in A normalized to a dose of 6 mg/kg of ddI).

concentrations between these formulations (Fig. 4). This ratio increases from 0.5 to 1.2 over 4 h for L-PEG and may reflect the slow recognition of the liposomes. In case of L-DSPC, this ratio gradually increases from 2 to 11 at the end of 4 h. The ddI concentrations in the spleen and the spleen to serum ratios were higher for L-DSPC compared to ddI alone.

ddI is rapidly cleared from the circulation and renal clearance accounts for 99% of the eliminated dose (Desormeaux et al., 1994; Ray et al., 1990). For ddI given alone the kidney had the highest levels and tissue to serum ratios. This would be consistent with renal elimination of this drug. Interestingly the total ddI levels in the kidney were higher with the L-DSPC formulation. On the other hand following L-PEG the kidney levels and tissue ratios of total ddI were the lowest of the three treatment groups. The ddI levels in the kidney in the first two groups are very high in the beginning which fall gradually as the drug is eliminated. In case of L-PEG, there was a higher amount of drug encapsulated (45%) and also due to the absence of a 5-min sample point any initial peak due to the rapid elimination of the unencapsulated drug was undetected.

One of the major adverse effects of ddI is its dose-dependent pancreatitis (Connolly et al., 1991; Kamali, 1993). From Fig. 3 it is seen that the ddI levels in the pancreas in all three treatment groups are similar ($p < 0.05$). A comparison of ratios however, shows L-PEG ratios in the pancreas to be lower than ddI alone or L-DSPC (Fig. 4). Desormeaux et al. (1994) have reported a possible reduction of pancreatitis by encapsulation of ddI in liposomes because of reduced accumulation in the pancreas in comparison to when ddI is administered as a solution. The results seen in this study show that the levels in the pancreas in all the three treatment groups are similar. But it may be possible to reduce the dose administered since the serum levels after L-PEG are high and consequently reduce the toxicity due to ddI.

In conclusion, encapsulation of ddI in DSPC:Chol liposomes improves its pharmacokinetic profile and alters ddI distribution. Coating these liposomes with PEG further decreases the clearance and reduce the accumulation in several tissues and may thus act as a better drug delivery

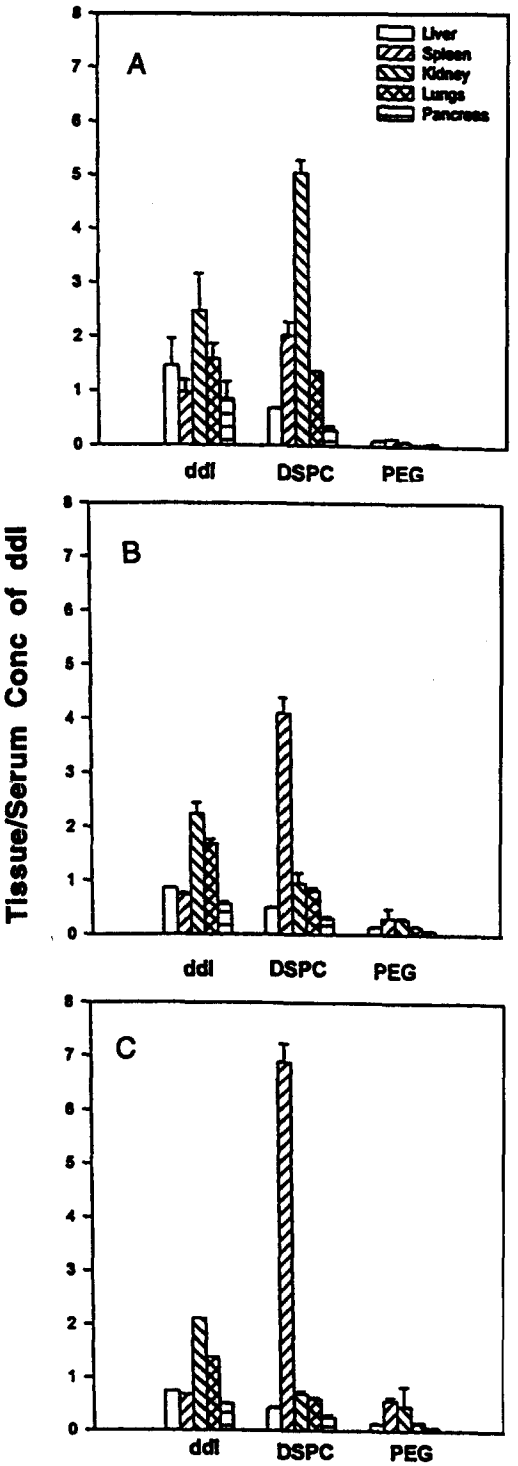

Fig. 4. Ratios of tissue to serum concentrations for total ddI: (A) 15 min after dosing; (B) 60 min after dosing; and (C) 120 min after dosing.

system to enhance the beneficial effects of the drug and at the same time reduce the toxic effects. Encapsulation of ddI in liposomes as well as PEG-liposomes deliver significant amount of drug to liver and spleen which are major targets because these organs contain significant number of macrophages which act as reservoir for HIV. Also, all CD4 antigen bearing cells are targets. By increasing the residence time of ddI in the vascular compartment provides an opportunity to deliver the drug to other CD4 antigen bearing cells, resident macrophages and microglia in the brain since PEG-liposomes have the ability to cross the blood-brain barrier. As noted earlier the toxicity of ddI is dose related, encapsulation of ddI in PEG-liposomes provides an excellent alternative towards reducing the dose and thus improve the therapeutic effectiveness of ddI.

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