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PHARMACOKINETICS OF LIPOSOME-ENCAPSULATED ANTI-TUMOR DRUGS

STUDIES WITH VINBLASTINE, ACTINOMYCIN D, CYTOSINE ARABINOSIDE, AND DAUNOMYCIN

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Abstract We have investigated the effects of encapsulation within liposomes (phospholipid vesicles) on the plasma clearance kinetics and tissue disposition of four anti-tumor drugs, namely vinblastine, cytosine arabinoside, actinomycin-D and daunomycin. In each case, subsequent to intravenous administration, the liposome-encapsulated drugs were cleared from the plasma much more slowly than were the free drugs. For example, the major portion of daunomycin injected in free form had a plasma half-life of less than 5 min, while liposome-encapsulated daunomycin had a plasma half-life in excess of 150 min. Encapsulation also caused a marked alteration in the tissue disposition of the injected drugs. Thus, encapsulation within liposomes resulted in a large increase in the total amount of drug equivalents retained by the tissues at various times after injection. In the case of cytosine arabinoside, for example, the level of drug equivalents in the liver at 16 hr post injection was 68-fold greater for liposome-encapsulated drug than for free drug. Encapsulation also altered the relative distribution of drugs in the tissues, with tissues rich in reticuloendothelial cells, such as liver and spleen, being the favored sites of uptake.

A rational approach to cancer chemotherapy requires a clear understanding of the multiple pharmacodynamic factors which govern the amount of active anti-neoplastic agent reaching target sites within the tumor or sites within sensitive normal tissues [1]. Recently, attempts have been made to control the pharmacokinetics and disposition of drugs by means of "drug delivery systems" [2, 3]. One approach to controlled drug delivery is the use of liposomes (phospholipid vesicles) as carriers for drugs [4] or enzymes [5]. It has been demonstrated that encapsulation within liposomes can dramatically alter the plasma clearance and tissue disposition of a variety of drugs [4, 6–8]. Moreover, the size and surface charge characteristics of the liposomal carrier are important determinants of the fate of the encapsulated drug *in vivo* [6, 7, 9, 10]. Recent reports that liposome encapsulation enhances the anti-tumor action of actinomycin D [11], and that liposome–methotrexate is protected against metabolic degradation [8], suggest that encapsulated drug formulations may have a role in cancer chemotherapy. However, before an intelligent approach toward the therapy of neoplasms using encapsulated drugs can be made, more detailed information on the pharmacology of these agents is necessary. Therefore, we have investigated the effects of liposome encapsulation on the pharmacodynamics of several clinically significant [12] anti-tumor agents including vinblastine, daunomycin, actinomycin-D and cytosine arabinoside.

METHODS

Materials and animals. All experiments were performed with adult male rats weighing 280–350 g. Egg phosphatidyl choline (PC) was prepared as described [13], phosphatidyl serine (PS) and cholesterol were purchased from Applied Science Labs, and stearyl amine (SA) from K & K Laboratories. Tritium-labeled actinomycin-D (3 Ci/m-mole) and vinblastine sulfate (5–10 Ci/m-mole) were obtained from Amersham Searle. Tritiated cytosine arabinoside (5–15 Ci/m-mole) was purchased from New England Nuclear. These radiochemicals were at least 95 per cent pure as indicated by paper or thin-layer chromatography according to their suppliers. Non-radioactive drugs were obtained from the following sources: vinblastine, Eli Lilly Co.; actinomycin-D, CalBiochem; cytosine arabinoside (cytosine β-D-arabinofuranoside), Upjohn Co.; and daunomycin, Poulenc Pharmaceuticals, Montreal. Other chemicals were of reagent grade and were used as purchased. Radiation counting supplies (Liquifluor, Aquasol and Protosol) were obtained from New England Nuclear.

Preparation of liposomes. Liposomes containing encapsulated anti-tumor drugs were prepared largely according to methods previously described [6]. Briefly, lipids in organic solvents were evaporated under vacuum onto the wall of a glass tube. Lipophilic drugs such as actinomycin-D, vinblastine and daunomycin were dissolved in ethanol and mixed with the lipid prior to evaporation. In order to form liposomes, physiologically iso-osmotic phosphate-buffered saline was added to the dried lipid film, and the mixture was dispersed by vortex agitation and sonication. Polar drugs such as cytosine arabinoside were incorporated into the liposomes by adding them to the aqueous buffer. Sonic dispersion of the drug containing liposomes was carried out in a bath-type instrument (Heat systems 125 W) under a nitrogen atmosphere and with continuous temperature control; the usual temperature for preparation of liposomes

was 35 , and the sonication period was typically about 18 hr. As described previously [14], unsonicated liposomes are large multilamellar structures, while extensively sonicated liposomes consist primarily of small vesicles bound by a single bimolecular lipid membrane.

Liposomes containing encapsulated anti-tumor drugs were separated from free drug by filtration on a Sephadex G-50 column. The void volume fraction was found to contain all the lipid plus encapsulated drug while the free drug was retarded by the column. The encapsulation or trapping efficiency was estimated from the relative size of the void volume and retarded volume peaks. Drug containing liposomes were usually further processed by pressure ultrafiltration in an Amicon cell using an XM-100A membrane followed by centrifugation at 10,000 rev/min for 20 min. The ultrafiltration served to remove the last traces of free drug and to concentrate the sample, while the centrifugation removed most residual, large multilamellar liposomes, leaving a preparation of mainly small liposomes containing entrapped anti-tumor drug, and devoid of free drug (see Fig. 1).

Clearance and disposition studies. Anti-tumor drugs either in free or liposome-encapsulated form were given to rats by tail vein injection. In the cases where free drugs were used, 5–50 μg of the agent was injected per animal, while usually 1–5 μg of encapsulated drug was used (the total amount of drug plus lipid injected was kept at a low level so as not to saturate the system responsible for liposome clearance [5]. In most cases the specific activity of the injected drug was in the range of 0.1 to 1.0 μCi/μg. For determination of the elimination rate from the blood, plasma samples were taken at 2 min post injection and periodically thereafter. Plasma levels of tritium or fluorescence (for daunomycin) were determined and expressed as the per cent of the initial level. For determination of tritium, 100-μl plasma aliquots were counted in Aquasol. Drug half-lives were estimated from semilog plots of the plasma clearance data by extrapolation of linear segments of the clearance curves.

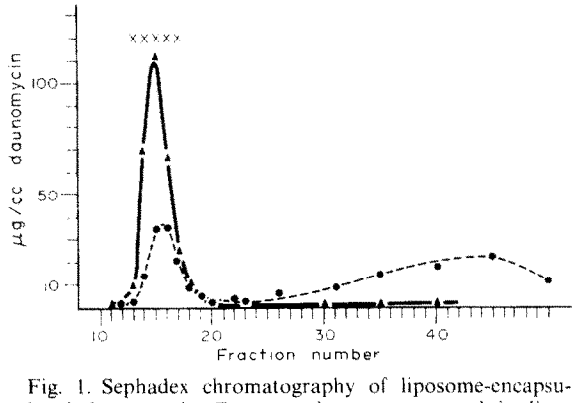

Fig. 1. Sephadex chromatography of liposome-encapsulated daunomycin. Daunomycin was entrapped in liposomes composed of PC, SA and cholesterol, and free and encapsulated drug were separated on a Sephadex G50 column. The void volume peak which contained all the lipid (cross hatching) and the encapsulated daunomycin was concentrated by pressure ultrafiltration and re-chromatographed. Key: (●–●) encapsulated plus free daunomycin; and (▲–▲) re-chromatography of void volume peak after concentration.

The tissue disposition of the administered drugs and their products were determined at 3 and 16 hr post injection. The following tissues were taken: brain (cerebrum), spleen, kidney, heart (whole organ), liver and lung (two lobes each), intestine (duodenum) and skeletal muscle (thigh muscle). Samples were disrupted with a Polytron homogenizer (Kinematia Gmbh) in 1–2 cm^3 water. Aliquots (100 μl) were taken for radiation counting or determination of fluorescence and, after appropriate dilution, for protein assay via the Lowry–Folin reaction [15].

Radioactive samples (vinblastine, actinomycin-D and cytosine arabinoside) were digested overnight at 60° with 1 cm^3 Protosol, then 10 cm^3 toluene Liquifluor Protosol (10:1:1) was added and samples were maintained at room temperature in tightly closed vials for approximately 2 weeks to allow for decay of chemiluminescence. The samples were counted in a Packard liquid scintillation counter with automatic external standardization.

Samples containing daunomycin were analyzed by the fluorometric technique of Bachur *et al.* [16]. Briefly, tissue homogenates were extracted with acid ethanol, centrifuged, and the clear supernatant fluid was saved for fluorometric assay. The assay was performed using an Aminco–Bowman spectro-photofluorimeter, and calibrated using known quantities of daunomycin dissolved in acid ethanol. Samples were excited at 470 nm and the emission was measured at 560 nm. The fluorescence spectra of samples were occasionally monitored to insure that they conformed to the spectrum of a daunomycin standard.

Data analysis. Analysis of the tissue disposition of tritium-labelled anti-tumor drugs was performed in two ways. For evaluation of the relative distribution of drug, i.e. the tendency to accumulate in one organ or another, the following approach was used. Results were initially expressed as ^{3}H dis./min/mg of tissue protein; since in various experiments rather different amounts of isotope were used, the data were normalized by assigning a value of 100 per cent to the tissue with the highest uptake (dis./min/mg) in a particular experiment, and calculating the relative accumulation in other tissues on a scale of 0–100 per cent on this basis. Calculated percentages were then averaged over the several experiments performed.

For comparison of the total amount of drug retention in tissues after administration of free or encapsulated anti-tumor drugs, the following analysis was used. Measured values of tritium dis./min or fluorescence intensity were converted into μg of drug equivalents based on the known specific activity or fluorescence intensity of the compounds administered. This analysis does not discriminate between intact drug and its radioactive (or fluorescent) breakdown products. Results were calculated in terms of μg of drug equivalent per gram of tissue protein per mg of injected drug.

The blood contribution to drug levels in tissues was evaluated using the measured values of plasma drug levels at sacrifice, and values for organ blood volumes as determined by infusion of isologous ^{51}Cr-labeled erythrocytes, as described previously [9]. Only in the case of liposome cytosine arabinoside was the blood

contribution appreciable (20 per cent of total) and the data were corrected accordingly.

RESULTS

Formation and stability of liposome–drug complexes. The drugs used in this study differed widely in chemical structure and physical properties. This is exemplified by their octanol–water partition coefficients seen in Table 1. Cytosine arabinoside is a highly polar drug and exhibits a strong preference for the aqueous phase, while the other three drugs partition mainly into the organic phase. In terms of the formation of drug–liposome complexes, it seems likely that cytosine arabinoside would be restricted to the internal aqueous compartment of the liposome, while the other drugs may also intercalate into the hydrocarbon region of the liposome membrane.

The chemical properties of the lipids themselves might also be expected to influence the formation of drug–liposome complexes. This is illustrated in Table 2, where one can see that the presence of cholesterol and of positively charged (stearyl amine) lipid promote the efficient incorporation of either actinomycin-D or cytosine arabinoside into liposomes. With this in mind, further experiments were conducted with positively charged, cholesterol-containing vesicles, with a molar ratio of 20:10:2 (PC–cholesterol–SA).

The ability of liposomes to trap or encapsulate several types of anti-tumor drugs is seen in Table 3.

Table 1. Octanol–water partition coefficients for anti-tumor drugs*

Drug	Partition coefficients (N = 3, 4)
Cytosine arabinoside	0.0012 ± 0.0002
Daunomycin	4.6 ± 0.9
Actinomycin-D	90.8 ± 0.9
Vinblastine sulfate	92.5 ± 2.0

* Partition coefficients were determined as follows. A 100-μg aliquot of drug was added to 2 ml octanol plus 2 ml of isotonic phosphate buffer (pH 7.2). The samples were thoroughly mixed and the phases separated by centrifugation. Aliquots (100 μl) were removed from the upper and lower phases and analyzed for radioactivity or fluorescence (daunomycin). The partition coefficient was taken as the ratio of the amount of drug in the upper (octanol) phase over the amount of drug in the lower (buffer) phase. Drug recoveries were greater than 90 per cent.

*The fraction of encapsulated cytosine arabinoside (0.5 to 1.2 per cent) is consistent with simple entrapment of the drug in the aqueous compartment of the liposomes. If we assume an average diameter of 300 Å for liposomes, a molecular weight of 800 for phosphatidyl choline and a trapped volume of 1.0 μl/μmole of phospholipid [17], then the amount of phospholipid used in the studies shown in Table 3 (4 mg) would entrap ~ 5 μl. The actual amount of entrapped cytosine arabinoside represents 10–20 μl of a total of 2.0 cm^3 of suspending buffer. Since we know that at least a portion of our liposome preparation consists of particles larger than 300 Å, we would expect that 4 mg phosphatidyl choline could entrap somewhat more than 5 μl, and thus the calculated and observed encapsulation efficiencies are not different by more than a factor of two or three.

Table 2. Effect of liposome composition on drug encapsulation*

Drug	Liposome PC	SA	PS	Chol	Per cent of incorporation
Cytosine arabinoside	+				0.19
	+			+	0.50
	+	+			0.35
	+	+		+	1.30
	+		+		0.09
	+		+	+	0.80
Actinomycin-D	+				15.3
	+			+	19.3
	+	+			34.0
	+	+		+	50.5
	+		+		11.6
	+		+	+	20.0

* Liposomes were prepared from PC (4 mg) with the addition of either SA or PS (0.4 mg) and of cholesterol (0.5 mg). A trracer dose of either [^{3}H]cytosine arabinoside or [^{3}H]actinomycin-D was included during liposome preparation. Free and liposome-encapsulated drugs were separated as described in Methods and the per cent of the input dose present as liposome-encapsulated material was calculated.

The trapping efficiency of the very polar drug cytosine arabinoside is low, and is probably related to the fraction of the total aqueous phase enclosed during liposome formation [14].* Nonetheless, substantial amounts of this drug can be encapsulated because of its high solubility in water. Non-polar drugs such as actinomycin-D are entrapped in liposomes with high efficiency. Although the percentage of entrapped drug declines with increasing input doses, a very substantial amount of actinomycin-D as well as of vinblastine and daunomycin can be entrapped.

The stability of liposomes containing anti-tumor drugs was evaluated by measuring the efflux rates of

Table 3. Efficiency of drug incorporation into liposomes*

Drug	Amount (μg)	Per cent incorporation	Incorporation (μg/mg PC)
Actinomycin-D	1.0	66.0	0.16
	50.0	71.0	8.8
	500.0	28.0	35.0
Cytosine arabinoside	2.0	0.60	0.003
	200.0	1.15	0.57
	2×10^4	0.52	26.5
Daunomycin	400.0	30.0	30.0
	1000.0	23.0	58.0
Vinblastine	25.0	11.0	1.4

* Various doses of drug were incorporated into liposomes composed of PC, SA and cholesterol (20:2:4 by weight). Usually 4 mg PC was used per preparation. In the cases of actinomycin-D, vinblastine and daunomycin, the drug was dissolved in ethanol and added to the lipid mixture prior to evaporation. In the case of cytosine arabinoside the drug was added in 2 cm^3 of buffered saline to the dried lipid film. The per cent of incorporation was measured by separation of free and liposome-bound drug on a Sephadex G-50 column as described in Methods. Results are the means of duplicate determinations.

Table 4. Efflux rates of liposome-encapsulated drugs*

	Efflux (per cent/hr) (N = 3)		
	Hr 1	Hr 2	Hr 3
Cytosine arabinoside	4.9 ± 0.3	4.1 ± 0.5	5.9 ± 1.0
Daunomycin	0.93 ± 0.0	0.37 ± 0.04	0.22 ± 0.01
Actinomycin D	0.71 ± 0.15	0.87 ± 0.07	1.06 ± 0.46
Vinblastine sulfate	0.45 ± 0.01	0.32 ± 0.03	0.20 ± 0.01

* Drugs (150 μg) were encapsulated in monolamellar liposomes composed of PC, SA and cholesterol. Efflux of drugs from the liposomes into buffer solutions at 25° was measured by the dialysis bag technique as described elsewhere [18]. The efflux rates were determined by measurement of radioactivity or fluorescence (daunomycin). The addition of serum up to 50 per cent had no effect on the efflux rates.

entrapped drugs. As seen in Table 4, loss of the three non-polar drugs from the liposomes was extremely slow (less than 1 per cent/hr) while the efflux of cytosine arabinoside was somewhat more rapid (4–5 per cent/hr). These results suggest that, with the possible exception of cytosine arabinoside, the drugs tested form a tight association with the liposome carrier and that their disposition *in vivo* should be strongly influenced by the disposition of the carrier. It is interesting to note that the efflux rate of the hydrophilic drug methotrexate from positively charged liposomes was less than 1 per cent/hr [8]. The difference between this value and that observed for cytosine arabinoside in the present study may be due to the fact that dianions such as methotrexate are likely to permeate lipid membranes more slowly than uncharged species such as cytosine arabinoside [19].

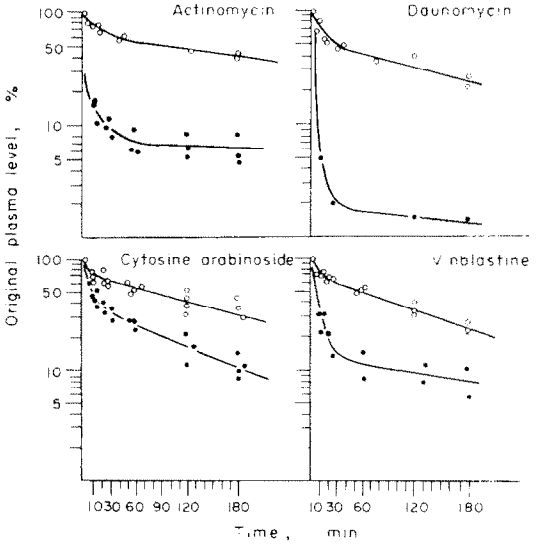

Fig. 2. Plasma clearance kinetics for free and liposome-encapsulated drugs. Rats were injected via the tail vein with free or encapsulated drug. At 2 min post injection, and at times thereafter, blood samples were obtained by retro-orbital puncture and the radioactivity or fluorescence in the plasma was analyzed. Open circles, encapsulated drug; and closed circles, free drug.

Plasma clearance of free and encapsulated drugs. The effects of liposome encapsulation on the plasma clearance kinetics of four anti-tumor drugs are illustrated in Fig. 2. In all cases encapsulation within liposomes markedly retarded the removal of the drug from the circulation, in agreement with previous studies [4, 6, 8]. The clearance curves for the liposome-bound drugs were all biphasic and showed considerable resemblance to each other. Thus a portion of the liposome-encapsulated drug (10–40 per cent) was cleared rather rapidly ($T_{1/2}$ ~ 8–10 min), while the bulk of the drug was cleared quite slowly ($T_{1/2}$ ~ 150–200 min). From previous experiments [6] it seems possible that the rapid phase of clearance is due to the removal of residual multilamellar liposomes from the preparation, while the slow phase represents the clearance of smaller unilamellar vesicles; however, at present, we cannot rule out other explanations of the observed clearance rates.

The clearance curves for the free drugs tended to resemble those previously described in the literature. Thus actinomycin-D had a major rapid phase of clearance ($T_{1/2}$ ~ 2 min) followed by a much slower phase ($T_{1/2}$ ~ 3 hr) [4]. Daunomycin has also been reported to be rapidly cleared from rats when given in free form [20], and in our hands the half-time for the major initial phase of clearance was less than 2 min. Although vinblastine has been reported to bind to serum proteins [21] and to platelets [22], the bulk of this compound was cleared rapidly ($T_{1/2}$ < 5 min)

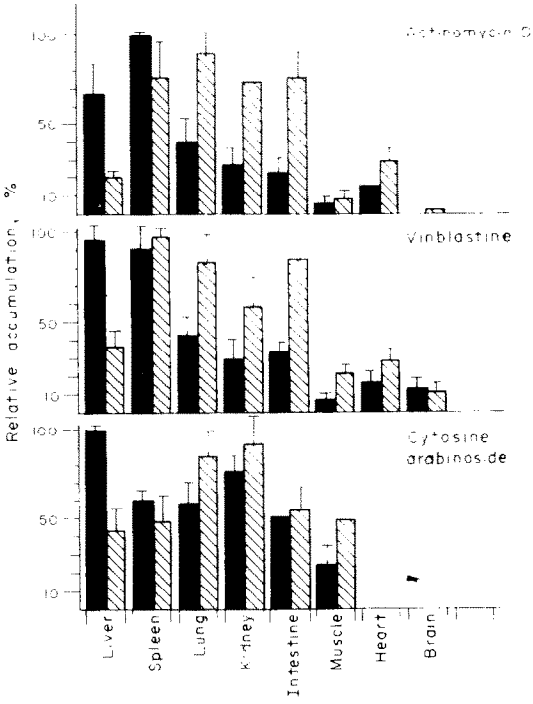

Fig. 3. Relative accumulation of ^3H after injection of free or encapsulated ^3H anti-tumor drugs. Animals were killed at 3 hr after injection of radiolabeled free or liposome-encapsulated drug, and organ samples were removed and processed as described in Methods. The data represent the means and standard errors for three to five animals (two animals where standard errors are not shown). Key: liposome-encapsulated drug, solid bars; and free drug, cross-hatched bars.

Table 5. Tissue accumulation of drug equivalents at 3 hr post injection*

Tissue	Actinomycin D			Cytosine arabinoside		
	A (free)	B (encapsulated)	B/A	C (free)	D (encapsulated)	D/C
Liver	25.0	312.0	12.5	8.0	154.0	19.2
Spleen	99.0	500.0	5.1	9.0	92.0	10.2
Lung	149.0	222.0	1.5	20.0	77.0	3.9
Kidney	57.0	211.0	3.7	16.0	120.0	7.5
Intestine	127.0	298.0	2.3	9.0	96.0	10.6
Muscle	14.0	38.0	2.4	6.0	38.0	6.3
Heart	46.0	158.0	3.4			

* Units = (μg drug equivalents)/g tissue protein/mg injected drug).

The μg values of drug equivalents were calculated from the known specific activities of the injected drugs and the measured dis./min in the tissues. The results represent total accumulation and do not discriminate between parent drug and radiolabeled degradation products. The data represent the means of two to three animals for each set of determinations.

from the rat blood stream (Fig. 2), or from the dog bloodstream [23], when given in free form. In the case of cytosine arabinoside, about half the material present initially was rapidly cleared while the remainder was cleared with a $T_{1/2}$ of approximately 2 hr. This situation seems to resemble the results observed in mice where a bi-phasic but relatively rapid clearance was observed [24]. In the present study we have not evaluated what proportion of the radioactivity found in the plasma with either the free or encapsulated drugs represents the parent compounds as opposed to metabolites [25].

Tissue disposition of free and encapsulated drugs. Figure 3 illustrates the relative accumulation of ^{3}H after the injection of free or encapsulated ^{3}H antitumor drugs. Our results agree qualitatively with previous reports [4, 7, 8] in that the favored sites of accumulation for encapsulated drug seemed to be tissues rich in reticuloendothelial cells such as liver, spleen and lung. However, earlier reports on liposome uptake by tissues [4, 5] suggested that the liver and spleen were overwhelmingly the preferred uptake sites while we found considerable accumulation in other tissues as well. These earlier studies used multilamellar liposomes and expressed their results as per cent injected dose per organ. In current studies we have expressed the level of accumulation in the tissues in terms of dis./min/mg of tissue protein; thus our data are independent of organ size. In addition, in a previous study, we have shown that unilamellar liposomes have a broader tissue distribution than do large multilamellar liposomes [9]. These considerations may explain the differences between earlier studies and current observations.

While in the cases of vinblastine and actinomycin-D, marked differences in tissue distribution were observed (Fig. 3) depending on whether the drug was given in free or encapsulated form, in the case of cytosine arabinoside the liver is the only tissue where a significant difference was seen in the relative accumulation of free and encapsulated drug. At present no explanation can be given for the observation that cytosine arabinoside in encapsulated form is more widely distributed in the viscera than are actinomycin-D and vinblastine. Present results on the distribution of free drug are consistent with previously published work. Thus the favored sites of uptake of free actinomycin-D include spleen, intestine and kidney [26, 27], while favored sites for free vinblastine include spleen, intestine and lung [28].

In addition to alteration of the relative disposition of drug, liposome encapsulation has the effect of enhancing the total amount of drug accumulated by various tissues. Thus in Table 5 it can be seen that at 3 hr post injection, the total amounts of actinomycin-D or cytosine arabinoside drug equivalents in the various tissues were from 2- to 20-fold higher when the drug was given in encapsulated form than was the case for free drug. Once again, as in Fig. 2, the preferred sites of uptake were clearly reticuloendothelial-rich organs such as liver and spleen. In the case of cytosine arabinoside a substantially higher accumulation of encapsulated drug was seen in other organs as well. Preliminary experiments with daunomycin indicated that liposome encapsulation also enhanced the accumulation of this drug in various tissues by about a factor of ten. These observations are consistent with previous studies on the tissue retention of free versus encapsulated EDTA and actinomycin-D [7, 26].

Differences in the levels of drug equivalents accumulated in the tissues were even more pronounced at long intervals after injection than at earlier points. Thus in Table 6 it can be seen that the accumulation of encapsulated cytosine arabinoside equivalents in

Table 6. Tissue accumulation of drug equivalents at 16 hr post injection*

Tissue	Cytosine arabinoside		
	A (free)	B (encapsulated)	B/A
Liver	2.3	158.0	68.7
Spleen	4.4	122.0	27.7
Lung	2.6	48.0	18.5
Kidney	2.9	57.0	19.6
Intestine	2.5	55.0	22.0
Muscle	2.6	50.0	19.2
Heart	2.9	62.0	21.3
Brain	3.7	62.0	16.8

* Units = (μg drug equivalents)/(g tissue protein/mg injected drug). Results represent the mean of two to three animals for each set of determinations.

various tissues was many-fold higher than the accumulation of free drug. In fact, a comparison of Tables 5 and 6 indicates that very little loss of encapsulated cytosine arabinoside occurred between 3 and 16 hr post injection, especially in the case of the liver. It is interesting to note that despite the relatively rapid efflux of cytosine arabinoside from liposomes (Table 2), encapsulation still has a marked effect on the retention of this drug in the tissues. Apparently biological removal processes such as renal clearance, and metabolic degradation of free drug may be more rapid events than drug efflux from the liposomes accumulated in the tissues. It should be remarked that, if the efflux rate in the tissues is the same as that *in vitro* (namely ~5 per cent/hr, Table 4), then even at 16 hr post injection, the liposomes will retain ~44 per cent of their original burden of cytosine arabinoside in encapsulated form.

DISCUSSION

Ideally one would like to be able to deliver potent anti-neoplastic drugs primarily to tumor cells, with little uptake by sensitive normal cells. With this goal in mind, attempts have been made to promote specific liposome–cell attachment *in vitro* [29, 30]. However, there have been no reports thus far of successful "targeting" of liposomes to tumor cells *in vivo* [31]. Nonetheless it seems probable that one may be able to exploit the intrinsic properties of liposomes in order to enhance the efficacy of anti-tumor drugs. Some of the properties of liposome-encapsulated drugs and approaches to their exploitation in therapy are discussed below.

Encapsulation of a drug within liposomes alters the relationship of the drug to cell membrane barriers. This seens to offer some interes..ng possibilities for the therapy of certain drug-resistant neoplasms. Frequently tumor cells become resistant to drugs by altering their membrane permeability barriers [32]. While drugs usually enter cells via passive or carrier-mediated diffusion, liposomes enter either by fusion or endocytosis [33]. Exploiting these differences in the route of uptake, Poste and Papahadjopoulos [34] were able to overcome the permeability barrier of actinomycin-D-resistant tumor cell mutants and obtain effective cell killing by encapsulation of actinomycin-D within liposomes. It seems likely that this approach may be generalized in connection with many types of tumors where resistance is due to permeability changes.

Many anti-tumor agents have rather short biological half-lives due to rapid metabolic inactivation, rapid excretion or both [1]. As described in this paper (Fig. 2, Tables 5 and 6) and elsewhere [4, 8, 9, 26] liposome encapsulation prolongs the plasma lifetime and increases the tissue retention of several anti-tumor drugs. This may be due to reduced renal clearance [9] and to reduced metabolic degradation [8, 9], as well as to the promotion of drug entry into cells, as described above. These effects may be especially important in enhancing the efficacy of drugs which act during the S-phase of the cell cycle. It seems clear that, for rapidly growing tumors, optimal therapeutic effects are obtained with S-phase specific drugs when there is continuous exposure of the tumor cells to the drug for a period of greater than two generation times [35]. Administration of S-phase specific drugs (such as cytosine arabinoside) in liposome-encapsulated form may allow the establishment of therapeutically effective tissue levels for long periods without the necessity of continuous drug infusion. A potential problem with this approach, however, is the question of whether liposome-encapsulated cytosine arabinoside would be efficiently anabolized to form cytosine arabinoside triphosphate [25], the active form of this drug. Recent results on the efficacy of liposome cytosine arabinoside against the L1210 mouse leukemia [36] would indicate that at least some of the encapsulated drug can be anabolized and so kill the tumor.

Liposome encapsulation not only enhances the total amount of drug accumulated in the tissue, but also alters the relative distribution of drug between different tissues (Fig. 3). Thus in most instances, liposome encapsulation results in enhanced accumulation in reticuloendothelial cell-rich tissues such as liver and spleen, and also in reduced accumulation in the gut, kidney and cardiac and skeletal muscles. These consequences of liposome encapsulation may offer some therapeutic advantages in cases where the utility of a drug is limited by its toxicity to the gastrointestinal tract, or by nephorotoxicity or cardio-toxicity.

In summary, encapsulation of anti-tumor drugs within liposomes markedly alters the pharmacodynamic characteristics of these drugs. Encapsulation results in prolonged plasma lifetimes, in enhanced levels of drug accumulation in the tissues, and in altered patterns of tissue disposition for several clinically significant anti-tumor agents.

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