Eur J Cancer Clin Oncol, Vol. 20, No. 8, pp. 1105–1112, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00
Pergamon Press Ltd.

The Treatment of Intravenously Implanted Lewis Lung Carcinoma with Two Sustained Release Forms of 1-β-D-Arabinofuranosylcytosine*

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Abstract—*Liposomes have been used in recent years as carriers for drugs and molecules of biological importance. In cancer chemotherapy, however, the advantages of liposome encapsulation of antitumor drugs remain uncertain, with the possible exception of the usefulness of encapsulated 1-β-D-arabinofuranosylcytosine (ara-C), an antitumor drug of a very short half-life. Liposome-encapsulated ara-C has been shown by others to enhance significantly the survival time of mice bearing leukemia, and the enhancement may be attributable to the role of liposomes as a slow release system for ara-C. We now further explore the advantages of two sustained release systems for ara-C, namely the liposome-encapsulated ara-C and 1-β-D-arabinofuranosylcytosine-5'-diphosphate-L-1,2-dipalmitin (ara-CDP-L-dipalmitin, a prodrug of ara-C). Intravenously implanted Lewis lung carcinoma is used as a solid tumor model. The therapeutic effectiveness of the two slow release forms of ara-C given by either i.v. or i.p. injections is examined. Viable tumor cells (1.0×10^5 cells/mouse) were inoculated i.v. and treatment was initiated 24 hr later using three schedules of multiple treatments for liposomal ara-C and single or multiple injections of ara-CDP-L-dipalmitin. Liposomal ara-C given by the i.p. route consistently increased the number of cures (>120 days survival). For example, when nine small doses (10 mg/kg) were given on consecutive days by i.p. injections, 50% of mice given liposomal ara-C were cured, compared with 10% cures in the group given ara-C liposomes by i.v. and no cures in mice receiving free ara-C given according to the same schedules. On the other hand, ara-CDP-L-dipalmitin given at a single dose is more effective than an equal dose divided in five injections. However, no cures have been obtained by treatments with ara-CDP-L-dipalmitin. These results have further demonstrated the advantage of lipsomes as carriers for antitumor drugs of short half-life.*

INTRODUCTION

LIPOSOME-ENCAPSULATED drugs have been proven significantly more effective than conventional drug forms in experimental therapy of certain parasitic diseases [1, 2] and metal poisonings [3–5], but only limited improvement has been obtained by liposome encapsulation of antitumor drugs. However, for one of the antitumor drugs so far investigated, 1-β-D-arabinofuranosylcytosine (ara-C), liposome encapsulation has been shown to enhance significantly the survival time of mice bearing L1210 leukemia [6, 7]. This increased antitumor activity of encapsulated ara-C can be attributed to its protection from rapid degradation in the blood and to its slow release from the liposomes.

To investigate the effectiveness of ara-C in 'slow release' systems against rapidly metastasizing solid tumors, we tested two different sustained release forms of the drug: liposome-encapsulated ara-C and 1-β-D-arabinofuranosylcytosine-5'-diphosphate-L-1,2-dipalmitin (ara-CDP-L-dipalmitin). The latter is a phospholipid covalent conjugate of ara-C [8, 9] that has been shown to act as a prodrug of ara-C in the treatment of

Accepted 2 March 1984.
*This work was supported by the National Cancer Institute grant No. CA-21556 and the U.S. Department of Energy under contract No. W-31-109-ENG-38.
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various leukemias in mice [9, 10]. We selected the murine Lewis lung carcinoma, a rapidly metastasizing tumor, as our solid tumor model. We designed the study to permit us to compare the relative therapeutic effectiveness of i.p. and i.v. injections. This was done in light of the findings of Parker *et al.* that a selective increase of liposome-encapsulated [^{14}C]-adriamycin concentration in the lymphatic system was achieved by administering the liposomes via the i.p. route [11].

The findings reported here deal with three aspects of our study: (a) the therapeutic effectiveness of ara-C in slow release forms in mice bearing a solid tumor (Lewis lung carcinoma); (b) investigation of the therapeutic effectiveness of the two forms of ara-C used; (c) comparison of the effectiveness between the i.p. and the i.v. routes of drug administration.

MATERIALS AND METHODS

Lipids and chemicals

L-α-Dipalmitoylphosphatidylcholine, cholesterol and cytosine-1-β-D-arabinofuranosylcytosine were obtained from Sigma Chemical Co., St. Louis, MO. Stearylamine was purchased from K & K Laboratories, Inc., Plainview, NY. Amersham Corp., Arlington Heights, IL was the source of [5-^{3}H]-cytosine-β-D-arabinoside ([^{3}H]-ara-C), sp. act. 24 Ci/mmol. Sephadex G-50 Fine (20–80 μm) was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Reagent-grade chemicals and spectroanalyzed solvents (Fisher Scientific Co., Pittsburgh, PA) were used in all experiments. Disodium salt of ara-CDP-L-dipalmitin (mol. wt. 1025) was synthesized as described elsewhere [8].

Preparation of liposomes containing [^{3}H]*-ara-C*

Multilamellar liposomes (MLV) containing [^{3}H]-ara-C were prepared as described by Kobayashi *et al.* [6]. A chloroform–methanol mixture (8:1 by vol.) containing 20 μmol of dipalmitoylphosphatidylcholine (PC), 15 μmol of cholesterol (CHOL) and 2 μmol of stearylamine (SA) was added to a 100-ml round-bottom flask; the mixture was then dried to a thin film in a rotary evaporator. One milliliter of an aqueous solution of ara-C (75 mg/ml in water) was added to the flask with immediate stirring at room temperature; large multilamellar liposomes containing ara-C were thus formed. The liposomes were separated from the non-encapsulated ara-C by centrifugation at 12,000 ***g*** for 10 min and washed twice in cold isotonic salt solution (75 mM KCl, 75 mM NaCl). The average percentage of ara-C incorporated in MLV was 17.2 ± 0.7% of the total amount of ara-C used.

The stability of the liposomes was determined by the dialysis method described previously [12]. Liposomes containing [^{3}H]-ara-C were dialyzed against saline at room temperature and aliquots of saline were removed for analysis of [^{3}H]-ara-C radioactivity in a Beckman LS-333 liquid scintillation counter. Liposomes containing ara-C were stable, with less than 5% of the encapsulated drug leaking out in 24 hr. Freshly prepared liposomes were used for each therapy experiment.

Preparation of ara-CDP-L-dipalmitin samples for in vivo evaluation

Because of the insolubility of ara-CDP-L-dipalmitin in aqueous solution [8, 9, 13], samples for *in vivo* testing were prepared by sonication of drug suspensions in sterile saline solution using a Kontes micro-ultrasonic cell disrupter at power setting 8. Each sample was sonicated at 37°C. A regimen of 20 sec on, 20 sec off, repeated 10 times, was used to minimize heating effects. Aliquots of this clear stock solution were stored at 5°C and were resonicated as above at 37°C immediately prior to the time of treatment. The structural and aggregational characteristics of ara-CDP-L-dipalmitin made up in this fashion have been described elsewhere [13].

Maintenance of Lewis lung carcinoma

C57BL/6 mice bearing subcutaneous Lewis lung carcinoma (LLC) were provided by the DCT Tumor Bank, Worcester, MA. LLC was maintained by serial passage of small (1 mm^3) tumor fragments into the subaxillary region of mice of the same strain. At day 14 after subcutaneous implantation the solid tumors were removed from the animals and minced in cold physiological saline solution under aseptic conditions. The tumor cell suspension was then filtered through a 253-μm-mesh nylon gauze. Cell viability was determined by the trypan blue dye exclusion method and a cell count was conducted in a hemocytometer under a light microscope.

Therapy experiments

Female mice of the BDF$_1$ (C57BL/6 × DBA/2) strain were selected for the study. All those used were 2–4 months old and weighed 25–30 g. Each mouse was intravenously inoculated with 10^5 viable LLC cells suspended in a 0.2-ml physiological saline solution. Administration of non-radioactive ara-C in various forms and dosages (determined according to Kobayashi *et al.* [6]) was initiated 24 hr after the tumor cells were implanted. Liposomal ara-C contained about 1 mg of total lipid per injection. The mice were checked twice daily and any deaths were recorded.

The moribund mice were killed and necropsied and tissues were taken for histopathological examinations.

Tissue distribution of [^{3}H]*-ara-C*

Postinjection distribution of non-encapsulated and liposome-encapsulated [^{3}H]-ara-C in various tissues of BDF_1 female mice was also studied. [^{3}H]-ara-C in either form and in a 0.2-ml saline solution (60,000–90,000 counts/min) was given to each mouse either by an i.v. or i.p. injection. Groups of four mice were killed at various intervals after injection and samples of liver, spleen, lungs and blood were removed and processed for radioactivity analysis as described below.

Preparation of mouse tissues

Liver, spleen, blood and lungs were taken for ^{3}H analysis. Blood from a jugular vein was collected with a capillary micropipette. Duplicate samples of 100 μl each were absorbed into a small ball of cotton and allowed to air dry before being wrapped in a filter paper (Whatman No. 1, 4.25 cm diam.). The spleen and lungs were removed, weighed, wrapped in a filter paper and air dried. Three slices of liver, each weighing between 150 and 200 mg, were also wrapped separately for analysis. The total liver activity was calculated as the mean counts per mg from the slices times the total liver weight. The slices were always taken from standard locations which preliminary determinations had shown to be representative of the whole [4].

Analysis of [^{3}H]*-radioactivity*

Each sample, wrapped in a filter paper, was pressed in a pellet press (Parr Instruments Co., Moline, IL). After automatic combustion in a Packard 305 sample oxidizer and addition of scintillation fluid containing 15% PPO (2,5-diphenyloxazole) and 1% bis-MSB (*p*-bis-*o*-methylstyrylbenzene) in toluene, each sample was analyzed for ^{3}H activity in a Beckman liquid scintillation counter, Model LS-200 B. The oxidation of each tissue sample was followed at least once by the combustion of an empty filter paper to remove the residual activity in the system. In the case of tissues known to contain high levels of radioactivity, several successive empty filter papers were oxidized. The radioactivity obtained from these filter papers was added to that of the preceding tissue sample. Calculation of the amount of radioactivity injected was based on analysis of triplicate samples of each injection solution taken with the injection syringe. These samples were prepared for radioanalysis in the same manner as the blood.

RESULTS

Antitumor activity of ara-C

Tables 1–3 show the survival times of tumor-bearing mice after i.v. or i.p. injection with non-encapsulated and liposome-encapsulated ara-C administered according to three different schedules. In most cases, regardless of the route of administration (i.p. or i.v.), the survival time of mice given non-encapsulated ara-C according to these schedules was not significantly different from the survival time of untreated control mice. There were no cures (120-day survivors) in either group. In contrast, the survival time of tumor-bearing mice given ara-C in the liposome-encapsulated form was significantly greater than that of the untreated controls and mice receiving non-encapsulated ara-C. In addition, among those mice administered the encapsulated drug, i.p. injection was significantly and consistently more effective than i.v. injection in producing cures.

Table 1. *Survival time of tumor-bearing mice given ara-C at a dose of* 25 mg/kg *on days* 1, 2 *and* 3*

Group	Method of drug injection	Mean survival time (days)†	Survival time of treated/controls × 100 (%)	Range of survival time (days)	Cures‡
Control	untreated	22.5 ± 3.8	100	18–30	0/10
Non-encapsulated ara-C					
	i.v.	25.5 ± 3.6	113	21–31	0/10
	i.p.	29.1 ± 6.8§	129	20–39	0/10
MLV-encapsulated ara-C					
	i.v.	40.3 ± 3.7§	179	36–47	0/10
	i.p.	38.5 ± 2.7§	171	35–44	2/10

*Tumor cells (1.0×10^5 cells/mouse) were inoculated i.v. and 24 hr later the first treatment was administered i.v. or i.p. Only dead mice were included in the calculation of survival time. Ten mice were in each group.

†Mean survival time ± standard deviation.

‡Mice surviving longer than 120 days.

§Statistically different from control (*t* test, $P < 0.05$).

Table 2. Survival time of tumor-bearing mice given ara-C at a dose of 25 mg/kg *on days* 1, 4 *and* 7*

Group	Method of drug injection	Mean survival time (days)†	Survival time of treated/ controls × 100 (%)	Range of survival time (days)	Cures‡
Control	untreated	21.8 ± 3.5	100	20–32	0/10
Non-encapsulated ara-C					
	i.v.	20.8 ± 1.9	100	18–25	0/10
	i.p.	22.8 ± 3.2	104	18–29	0/10
MLV-encapsulated ara-C					
	i.v.	37.6 ± 8.0§	172	28–49	2/10
	i.p.	45.2 ± 6.3§	207	36–53	4/10

*†‡See Table 1.
§Statistically different from control (t test, $P < 0.05$).

Table 3. Survival time of tumor-bearing mice given ara-C at a dose of 10 mg/kg *on days* 1, 2, 3, 4, 7, 8, 9, 10 *and* 11*

Group	Method of drug injection	Mean survival time (days)†	Survival time of treated/ controls × 100 (%)	Range of survival time (days)	Cures‡
Control	untreated	28.9 ± 5.3	100	23–35	0/10
Non-encapsulated ara-C					
	i.v.	27.7 ± 4.4	100	21–36	0/10
	i.p.	30.2 ± 2.4	105	27–34	0/10
MLV-encapsulated ara-C					
	i.v.	37.9 ± 10.0	131	28–51	1/10
	i.p.	48.0 ± 15.5§	166	30–67	5/10

*†‡See Table 1.
§Statistically different from control (t test, $P < 0.05$).

*Table 4. Survival time of tumor-bearing mice treated with ara-CDP-L-dipalmitin**

Group/dosage	Route of administration	Mean survival time (days)†	Survival time of treated/ controls × 100 (%)	Range of survival time (days)	Cures‡
Control	untreated	22.4 ± 3.0	100	18–29	0/26
Free ara-C:					
3 × 50 mg/kg§	i.p.	27.2 ± 3.0	121	21–31	1/10
ara-CDP-L-dipalmitin:					
5 × 25 mg/kg‖	i.v.	23.7 ± 2.4	106	21–28	0/10
	i.p.	26.3 ± 4.1	117	20–34	0/10
5 × 50 mg/kg‖	i.v.	24.5 ± 3.5	109	21–31	0/10
	i.p.	28.4 ± 5.2¶	127	22–40	0/10
1 × 125 mg/kg	i.p.	25.7 ± 3.6	115	21–33	0/10
1 × 250 mg/kg	i.p.	29.6 ± 4.8¶	132	25–37	1/10
1 × 395 mg/kg	i.p.	34.0 ± 5.5¶	152	28–43	0/10

*†‡See Table 1.
§Administered on days 1, 3 and 5.
‖Administered on days 1, 3, 5, 7 and 9.
¶Statistically different from control (t test, $P < 0.05$).

When a total dose of 75 mg/kg of liposome-encapsulated ara-C was divided into three injections, spaced every third day (days 1, 4, 7), there were two cures in the group of ten mice receiving liposomal ara-C by the i.v. route and four cures in the i.p. group (Table 2). On the other hand, when the three injections were given on three consecutive days (days 1, 2, 3) there were no cures in the i.v. group and only two in the i.p. group (Table 1). When nine smaller doses (10 mg/kg per injection) were administered on days 1, 2, 3, 4, 7, 8, 9, 10 and 11, 50% of the animals in the

i.p. group receiving encapsulated ara-C and 10% in the i.v. liposome group survived longer than 120 days. The death of all animals seemed to be caused by lung tumors, because necropsy of dead animals in all groups revealed massive tumors in the lungs and metastatic tumors in skeletal or cardiac muscles. No evidence of toxicity due to the liposomal lipids used for the ara-C encapsulation was observed either by gross anatomy at necropsy or by histopathological examination of tissues.

Antitumor activity of ara-CDP-L-dipalmitin

Table 4 shows data on the survival of mice bearing Lewis lung carcinoma after treatment with ara-CDP-L-dipalmitin. Similar to the results described above, the survival of mice given free ara-C i.p. was not significantly different from the survival of untreated control mice. Intraperitoneal injection of ara-CDP-L-dipalmitin resulted in an increased survival time of tumor-bearing mice compared to that of the untreated controls, but essentially no increase in cures. When the ara-CDP-L-dipalmitin was administered i.v., no appreciable increase in survival time was observed.

The data in Table 4 also indicate that when the prodrug was administered i.p., the survival times within a particular total dose level (i.e. 125 mg/kg or 250 mg/kg) were very similar, whether the total dose was divided into five separate injections administered on alternate days or whether it was delivered as a single bolus on day 1. Survival appeared to be related to the total amount of prodrug administered, with the best results being obtained with the highest doses. [In the single-dose experiments the % *T/C* (treated/ controls) was 114.7, 132.1 and 151.8 for total doses of 125 mg/kg, 250 mg/kg and 395 mg/kg respectively.] No evidence of drug toxicity was seen, either as indicated by the life-span of the animals or by gross anatomy at necropsy, even at the highest dose of 395 mg/kg.

Tissue distribution of [^{3}H]*-ara-C*

There was a major difference between the patterns of tissue (blood, spleen, lung and liver) distribution of [^{3}H]-ara-C in non-encapsulated and liposome-encapsulated forms (Table 5). Uptake of ara-C in its non-encapsulated form was generally the same whether the drug was administered by i.p. or i.v. injection. In contrast, the uptake levels of liposome-encapsulated [^{3}H]-ara-C were in general significantly higher when given by i.v. injection than when administered i.p. (Table 5). An exception to this was the blood level at 3 and 24 hr after injection, when the concentration of ara-C was higher in animals injected i.p. A prolonged tissue retention of ^{3}H-radioactivity was also observed in mice receiving the liposome-encapsulated form.

It is of interest that in mice receiving liposome-encapsulated ara-C by the i.p. route less than 0.5% of the injected dose was found in the lungs (the major tumor-bearing organ) at any time point (Table 5). Yet the therapeutic efficacy of the i.p. injection was superior to that of the i.v. injection (Tables 1–3).

Table 5. Distribution in mouse tissues of [^{3}H]*-ara-C (non-encapsulated or MLV-encapsulated) after i.v. or i.p. injection**

Group/tissues	Route of administration	15 min	30 min	1 hr	3 hr	24 hr
Non-encapsulated [^{3}H]-ara-C:						
Blood†	i.v.	4.75 ± 0.49	3.72 ± 0.28	2.25 ± 0.17	0.63 ± 0.12	-
	i.p.	4.99 ± 0.39	3.69 ± 0.18	3.21 ± 0.09	0.78 ± 0.10	-
Spleen	i.v.	0.27 ± 0	0.25 ± 0	0.16 ± 0	0.03 ± 0	-
	i.p.	0.37 ± 0	0.26 ± 0	0.23 ± 0	0.03 ± 0	-
Lungs	i.v.	0.57 ± 0.04	0.42 ± 0	0.22 ± 0	0.04 ± 0	-
	i.p.	0.55 ± 0	0.37 ± 0	0.25 ± 0	0.04 ± 0	-
Liver	i.v.	3.56 ± 0.07	2.69 ± 0.07	1.58 ± 0.08	0.34 ± 0	-
	i.p.	4.37 ± 0.07	1.99 ± 0.08	1.95 ± 0.08	0.30 ± 0	-
MLV-encapsulated [^{3}H]-ara-C:						
Blood†	i.v.	41.85 ± 6.11	27.51 ± 3.74	10.38 ± 1.29	0.86 ± 0.09	0.38 ± 0.02
	i.p.	1.96 ± 0.58	1.82 ± 0.66	2.63 ± 0.15	3.02 ± 0.60	2.34 ± 0.34
Spleen	i.v.	8.97 ± 0.52	12.42 ± 1.25	12.14 ± 1.15	9.93 ± 0.77	7.08 ± 1.04
	i.p.	0.20 ± 0.06	0.33 ± 0.12	0.58 ± 0.04	1.41 ± 0.49	1.70 ± 0.14
Lungs	i.v.	10.80 ± 0.53	8.33 ± 0.75	6.81 ± 0.54	3.27 ± 0.27	2.59 ± 0.19
	i.p.	0.21 ± 0.06	0.20 ± 0.06	0.26 ± 0.02	0.31 ± 0.07	0.26 ± 0.03
Liver	i.v.	25.69 ± 1.83	26.17 ± 0.81	34.20 ± 2.74	31.01 ± 0.92	27.26 ± 0.19
	i.p.	2.78 ± 0.89	3.45 ± 1.23	5.39 ± 0.49	8.48 ± 1.86	9.95 ± 1.84

*Values are mean percent of injected [^{3}H]-ara-C activity ± the standard error of the mean. Four mice were in each group.
†Activity in the estimated blood volume of 2 cm^3, as calculated from two 100-μl samples from each mouse.

DISCUSSION

While ara-C has proven useful in the clinical treatment of various types of leukemias, its use against solid tumors has not been known to be successful. However, three interesting findings emerge from our results: (a) when encapsulated in liposomes, ara-C is effective in inhibiting the growth of a solid tumor, i.e. Lewis lung carcinoma in mice; (b) ara-C in the liposome-encapsulated form cures up to 50% of mice bearing Lewis lung carcinoma; and (c) i.p. injection of both liposomal ara-C and ara-CDP-L-dipalmitin results in a better therapeutic efficacy of the drug than i.v. injection (Tables 1–4).

Due to the short half-life of ara-C, our data obtained from the tissue distribution study should be interpreted with caution. The radioactivity found in the tissues of mice given free or liposome-encapsulated [^{3}H]-ara-C would have to represent not only [^{3}H]-ara-C but its metabolites as well. The half-life of the liposome-encapsulated [^{3}H]-ara-C could, however, be expected to be significantly longer than that of the non-encapsulated form. In spite of this uncertainty, the results clearly show that i.p. injection of liposomes containing [^{3}H]-ara-C results in significantly lower uptake of liposomes in liver, spleen and lung (Table 5) compared to uptake after i.v. injection. This low uptake of ara-C in the lung after i.p. injection may rule out the mechanism of targeted drug delivery to the site of the tumor for the observed therapeutic improvement. The enhanced therapeutic efficacy of ara-C both in the liposome-encapsulated and phospholipid-derivative forms would thus seem to be best explained by the mechanism of a sustained drug release. With regard to the ara-CDP-L-dipalmitin, this hypothesis is in good agreement with previous work on L1210 lymphoid leukemia in mice in which the slow release of ara-CMP (or ara-C) was demonstrated [9].

Parker *et al.* have found that when administered by the i.p. route, liposome-encapsulated [^{14}C]-adriamycin is retained in the lymphatic system at a significantly higher level than that found in the plasma [11]. Based on those findings, it is reasonable to presume that the therapeutic activity of ara-CDP-L-dipalmitin and particularly of the liposome-encapsulated ara-C may also be due to an increased drug concentration in the lymphatics, although the underlying mechanism for this therapeutic effect is unclear. Lewis lung carcinoma is known to metastasize rapidly, making this tumor model a good choice for demonstrating the advantage of using liposomes as a sustained release system for antitumor drugs. Our data also show that a treatment schedule of smaller doses of ara-C encapsulated in liposomes given in eight or nine injections is not significantly superior to larger doses given in three spaced injections (cf. Tables 3 and 2). This finding is in contrast to the usual findings obtained with non-encapsulated ara-C.

In the case of ara-CDP-L-dipalmitin, it is of interest to compare the results of our study with those obtained previously with this type of phospholipid–nucleoside drug conjugate against various leukemias [9, 10, 14–16] and against human colon carcinoma HCT-15 in immunosuppressed mice [10]. As with the earlier studies, our results indicate that a single injection of the conjugate gives a similar therapeutic efffect as when the same dose is divided into five separate injections. This reinforces the concept that ara-CDP-L-dipalmitin is indeed acting as a slow release form of ara-C (or ara-CMP) [14]. Of particular interest is the apparent lack of toxicity of the ara-CDP-L-dipalmitin in this mouse strain (BDF_1, C57BL/6 × DBA/2), even at the single high dose of 395 mg/kg. This is in contrast to the observation that in $C3D2F_1J$ (C3h/HeJ × DBA/2J) mice ara-CDP-L-dipalmitin appeared to have some toxicity at levels of approximately 60 mg/kg/day × 5 (i.e. total dose 300 mg/kg). It should be noted that the latter strain is also somewhat more susceptible to ara-C toxicity than the former [14]. In previous studies against L1210 lymphoid leukemia ara-CDP-L-dipalmitin appeared to be slightly more effective when administered i.p. than when given i.m. (the i.v. route of administration was not examined) [14]. However, in the work described here the difference in effectiveness between i.p. and i.v. injection seems evident. In fact, little or no increase in life-span was observed when ara-CDP-L-dipalmitin was administered i.v. (see Table 4).

Overall the results described here indicate the usefulness of ara-CDP-L-dipalmitin as a sustained release form of ara-C, although the liposome-encapsulated form of ara-C, even on a molar basis, is clearly superior in this instance. It is important to note that ara-CDP-L-dipalmitin is an amphipathic molecule and exists in solution as large aggregates—the precise nature depending on the drug concentration, the ionic strength, the temperature and precise mode of sample preparation [13]. In some regards the supramolecular assemblies are similar to the liposomes described in this work (e.g. both are made up from amphipathic phospholipid-like molecules and form bilayer-type structures [13]). It should be noted, however, that ara-CDP-L-dipalmitin is anionic in character, while the multilamellar liposomes are made up from zwitterionic and basic amphiphiles.

Based on the number of mice cured (Tables

1–4), the difference in efficacy between the two sustained release forms of ara-C used in our study could be due to one (or more) of several factors, including (a) different mechanisms and rates of drug release (the ara-C or ara-CMP is released from the ara-CDP-L-dipalmitin by enzymatic processes [9, 10, 15], whereas the ara-C is released from the liposomes either by leakage or after slow destruction of the lipid bilayers of liposomes following cellular uptake) [6, 7]; (b) a different tissue distribution (confirmation of this point will have to be made by examining the tissue distribution of ara-CDP-L-dipalmitin when the labeled compound is available); (c) different rates of uptake of the ara-CDP-L-dipalmitin aggregates [13] relative to the liposome-encapsulated ara-C; or (d) different rates of metabolic activation of the drug to the active cytotoxic metabolite ara-CTP [17, 18] due to a different intracellular distribution of the released ara-C or ara-CDP-L-dipalmitin.

Acknowledgement—The authors thank Mr. John De Pue for his valuable assistance in the preparation of this manuscript.

REFERENCES

1. Alving CR, Steck EA, Chapman WL *et al.* Therapy of leishmaniasis: superior efficacies of liposome-encapsulated drugs. *Proc Natl Acad Sci* 1978, **75**, 2959–2963.
2. Alving CR, Schneider I, Swartz GM Jr, Steck EA. Sporozoite-induced malaria: Therapeutic effects of glycolipids in liposomes. *Science* 1979, **205**, 1142–1144.
3. Rahman YE, Rosenthal MW, Cerny EA. Intracellular plutonium: removal by liposome-encapsulated chelating agent. *Science* 1973, **180**, 300–302.
4. Rahman YE, Rosenthal MW, Cerny EA, Moretti ES. Preparation and prolonged tissue retention of liposome-encapsulated chelating agents. *J Lab Clin Med* 1974, **83**, 640–647.
5. Rahman YE. Potential of the liposomal approach to metal chelation therapy. In: Dingle JT, Jacques PJ, Shaw IH, eds. *Lysosomes in Biology and Pathology*. Amsterdam, Elsevier, 1979, 625–652.
6. Kobayashi T, Kataoka T, Tsukagoshi S, Sakurai T. Enhancement of antitumor activity of 1-β-D-arabinofuranosylcytosine by encapsulation in liposomes. *Int J Cancer* 1977, **20**, 581–587.
7. Rustum YM, Dave C, Mayhew E, Padahadjopoulos D. Role of liposome type and route of administration in the antitumor activity of liposome-entrapped 1-β-D-arabinofuranosylcytosine against mouse L1210 leukemia. *Cancer Res* 1979, **39**, 1390–1395.
8. MacCoss M, Ryu EK, Matsushita T. The synthesis, characterization, and preliminary biological evaluation of 1-β-D-arabinofuranosylcytosine-5′-diphosphate-L-1,2-dipalmitin. *Biochem Biophys Res Commun* 1978, **85**, 714–723.
9. Matsushita T, Ryu EK, Hong CI, MacCoss M. Phospholipid derivatives of nucleoside analogs as prodrugs with enhanced catabolic stability. *Cancer Res* 1981, **41**, 2707–2713.
10. Turcotte JG, Srivastava SP, Steim JM, Calabresi P, Tibbetts LM, Chu MY. Cytotoxic liponucleotide analogs. II. Antitumor activity of CDP-diacylglycerol analogs containing the cytosine arabinoside moiety. *Biochim Biophys Acta* 1980, **619**, 619–631.
11. Parker RJ, Hartman KD, Siever SM. Lymphatic absorption and tissue disposition of liposome-entrapped ^{14}C-adriamycin following intraperitoneal administration to rats. *Cancer Res* 1981, **41**, 1311–1317.
12. Lau EH, Cerny EA, Rahman YE. Liposome-encapsulated Desferrioxamine in experimental iron overload. *Br J Haematol* 1981, **47**, 505–518.
13. MacCoss M, Edwards JJ, Seed TM, Spragg SP. The aggregational characteristics and morphological aspects of selected 1-β-D-arabinofuranocylcytosine-5′-diphosphate-L-1,2-diacylglycerols. *Biochim Biophys Acta* 1982, **719**, 544–555.
14. Hong CI, MacCoss M, Ryu EK, West CR. 1-β-D-Arabinofuranosylcytosine conjugate of phospholipid as potential new antitumor prodrug. *Proc AACR* 1982, **23**, 200.
15. Raetz CRH, Chu MY, Srivastava SP, Turcotte JG. A phospholipid derivative of cytosine arabinoside and its conversion to phosphatidylinositol by animal tissue. *Science* 1977, **196**, 303–305.
16. Ryu EK, Ross RJ, Matsushita T, MacCoss M, Hong CI, West CR. Phospholipid–nucleoside conjugates. 3. The syntheses and preliminary biological evaluation of 1-β-D-arabinofuranosylcytosine-5′-monophosphate-L-1,2-dipalmitin and selected 1-β-D-arabinofuranosylcytosine-5′-diphosphate-L-1,2-diacylglycerols. *J Med Chem* 1982, **25**, 1322–1329.
17. Uchida K, Kreis W, Hutchison DJ. Distribution of 1-β-D-arabinofuranosyl-cytosine (ara-C) and enzyme profiles of P815 neoplasms. *Proc AACR* 1968, **9**, 72.

18. Uchida K, Kreis W. Studies on drug resistance—I. Distribution of 1-β-D-arabinofuranosyl-cytosine, cytidine and deoxycytidine in mice bearing ara-C-sensitive and resistant P815 neoplasms. *Biochem Pharmacol* 1969, **18**, 1115–1128.