

Liposomal Vincristine Which Exhibits Increased Drug Retention and Increased Circulation Longevity Cures Mice Bearing P388 Tumors¹

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Abstract

Prolonged exposure to vincristine correlates with improved therapeutic activity. In this work, two methods are used to increase the circulation longevity of liposomal formulations of vincristine. The first involves incorporation of the ganglioside G_{M1}, which acts to increase the circulation longevity of liposomal carriers, while the second approach relies on a modification of the vincristine encapsulation procedure which enhances drug retention. It is shown that these approaches are synergistic and increase the circulation half-life of vincristine from approximately 1 h to greater than 12 h. This results in a dramatic improvement in the therapeutic activity of liposomal vincristine as measured using a murine P388 lymphocytic leukemia model. At doses above 2 mg/kg, the optimized liposomal vincristine formulation cures greater than 50% of mice bearing the P388 tumor, whereas free vincristine results in no cures.

Introduction

Vincristine is a *Vinca* alkaloid derived from the periwinkle plant. It is an important anticancer drug that is effective against a wide variety of neoplasms including Hodgkin's and non-Hodgkin's lymphomas, acute lymphoblastic leukemia, embryonal rhabdomyosarcoma, neuroblastoma, breast carcinoma, and Wilms' tumor (1, 2). It is a cell cycle-specific drug which arrests cell growth exclusively during metaphase by attaching to the growing end of microtubules and inhibiting their assembly (3, 4). As a result, it is advantageous to expose neoplastic cells to the drug for prolonged periods of time. This has been demonstrated *in vitro* by Jackson and Bender (5) and has been confirmed in our laboratory using the murine L1210 leukemic cell line (6). The importance of this relationship in the treatment of human malignancies is supported by clinical trials where patients refractory to bolus vincristine therapy exhibited increased response rates when the drug was administered as a 5-day infusion (7, 8).

Previous work has shown that liposomal formulations of vincristine can exhibit reduced toxicity and enhanced efficacy compared to free drug (6, 9, 10) and indicated that the antitumor activity of vincristine is strongly dependent on the circulation lifetime of the encapsulated drug. The circulation longevity of liposomally entrapped vincristine is dependent on the circulation lifetime of the liposomal carrier and the rate of drug release from the liposomes in the blood. A strategy for improving the therapeutic activity of liposomal vincristine was developed based on these two parameters. Specifically, we have demonstrated *in vitro* that vincristine encapsulated in liposomes using the transmembrane pH gradient loading technique (9) is retained significantly longer in serum when the pH of the entrapped citrate buffer is initially 2.0 (11). Furthermore, it has been shown that incorporation of

the monosialoganglioside G_{M1} or PEG³-derivatized lipids in liposomes significantly enhances liposome circulation times (12-19). It is reported here that the effects of decreased pH_i and incorporation of G_{M1} act synergistically in combination to significantly enhance the circulation lifetime of encapsulated vincristine. The therapeutic activity of the resulting liposomal vincristine preparation is dramatically improved and results in cures (70-day survival) in over 50% of mice inoculated with P388 lymphocytic leukemia.

Materials and Methods

Oncovin (vincristine sulfate) was obtained from the B.C. Cancer Agency (Vancouver, British Columbia). DSPC was purchased from Avanti Polar Lipids and was greater than 99% pure. Monosialoganglioside G_{M1}, cholesterol, and all salts were obtained from Sigma Chemical Co. (St. Louis, MO). Cholesteryl hexadecyl ether (¹⁴C), a lipid marker that is not exchanged or metabolized *in vivo* (20), was synthesized by Amersham (Oakville, Ontario). Female BDF₁ mice (6-8 weeks old) were purchased from Charles River Laboratories (Ontario).

DSPC/Chol (55:45; mol:mol) or DSPC/Chol/G_{M1} (45:45:10; mol:mol:mol) solutions were prepared by dissolving the lipid mixtures in 95% ethanol (100 mg lipid/ml). The mixtures were then heated at 60°C for 30 min. Subsequently, a preheated (60°C) solution of 300 mM citric acid (pH 4.0 or 2.0) was added (100 mg total lipid/3 ml buffer) while vortex mixing vigorously. The resulting multilamellar vesicles were heated at 60°C for an additional 30 min to ensure equilibration of buffer across the vesicle membranes and were then extruded 10 times through two polycarbonate filters with 0.1 μm diameter pores (21). The extrusion device, obtained from Lipex Biomembranes (Vancouver, British Columbia), was also maintained at 60°C. Ethanol was removed from the liposome preparation by dialyzing (Spectra/Por 2 dialysis tubing, 12,000-14,000 MWCO) against two changes of 100 volumes of 300 mM citric acid (pH 4.0 or 2.0) over a 24-h period. More than 99.9% of the ethanol was removed using this procedure. Thin-layer chromatography analysis of all lipids, including G_{M1}, showed no degradation at either pH 4.0 or 2.0 (results not shown).

Vincristine was entrapped in the liposomes using the transmembrane pH gradient (ΔpH) loading procedure described elsewhere (9). Briefly, vesicles (25 mg/ml) were added to a vincristine solution (Oncovin; 1 mg vincristine/ml) to achieve a drug:lipid ratio of 0.1:1. The exterior pH of the liposome/vincristine mixture was raised to pH 7.0-7.2 with 0.5 M Na₂HPO₄ and immediately heated to 60°C for 10 min. High-performance liquid chromatography analysis (isocratic DEA:methanol mobile phase on a C8 column with spectrophotometric detection at 297 nm) of vincristine at both pH 2.0 and 4.0 showed no degradation over 24 h (results not shown).

Plasma clearance studies were performed by injecting 20 mg lipid/kg body weight of drug-loaded or empty liposomes via a lateral tail vein to female BDF₁ mice (18-22 g). The vincristine dose was, therefore, typically 2 mg/kg. Previous studies have shown that this dose of vincristine, when entrapped in liposomes, exhibits measurable levels of antitumor activity in L1210 and P388 ascites tumor models (9). Four mice were used per time point. The mice were anesthetized at the indicated time points (i.p. mixture of 160 mg/kg ketamine and 20 mg/kg xylazine). Blood was collected via cardiac puncture and placed into EDTA-coated microtainer tubes (Becton Dickinson). The samples were then centrifuged (500 × g for 10 min) to pellet the blood cells and obtain

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³ The abbreviations used are: PEG, polyethylene glycol; pH_i, interior pH.

plasma samples. Liposomal lipid and/or vincristine were then assayed using scintillation counting.

Biodistribution studies were performed on the same mice used for plasma clearance studies. Following heart puncture, animals were killed by cervical dislocation, and selected tissues were removed from each animal and weighed. Saline was added to each organ to achieve a 10% (w/v) ratio and homogenized using a Polytron homogenizer (Brinkmann Instruments, Rexdale, Ontario). Tissue homogenates (500 μ l) were digested with 500 μ l of "Solvable" (Du Pont, Inc.) for 3 h at 50°C. Subsequently, the samples were cooled to room temperature before decolorizing with 200 μ l of 30% hydrogen peroxide. Samples were then counted using Picofluor (Packard) scintillation cocktail. The statistical significance of both the plasma clearance and biodistribution results were determined using the Student *t* test.

The antitumor effects of liposomal vincristine were monitored using the P388 lymphocytic leukemia model. BDF₁ mice (5 per group) were injected i.p. with 1×10^6 cells. The indicated doses of saline or liposomal vincristine were administered (i.v.) 24 h after tumor inoculation. Animal weights and mortality were monitored daily. Selected treatment groups were repeated in order to achieve *n* values of 10. Mean and median survival times as well as the statistical significance of the results were determined using the Mann-Whitney-Wilcoxon procedure.

Results

Plasma Clearance and *in Vivo* Drug Release Studies. The influence of G_{M1} incorporation and entrapped vincristine on the circulation lifetime of 100-nm diameter DSPC/Chol liposomes is shown in Fig. 1A. Two important conclusions can be derived from this data: (a) the circulation lifetime of liposomes containing vincristine is greater than control, drug-free liposomes. This effect cannot be achieved by pretreatment with free drug (data not shown) and is consistent with previous results demonstrating that the presence of encapsulated doxorubicin significantly increases the circulation longevity of associated liposomal carriers (22, 23); (b) incorporation of 10 mol% G_{M1} in 100 nm DSPC/Chol liposomal vincristine results in a further increase in carrier circulation lifetime. Plasma liposomal lipid levels are increased approximately 2.5-fold 24 h after i.v. administration when G_{M1} is incorporated into DSPC/Chol liposomal vincristine as compared to DSPC/Chol. Liposomal lipid accumulation in liver and spleen (Fig. 1, B and C) is reduced significantly when DSPC/Chol/G_{M1} liposomes are used to encapsulate vincristine. This data is consistent with results which indicate that incorporation of G_{M1} decreases uptake of liposomes by phagocytic cells of the reticuloendothelial system (18, 24).

The influence of G_{M1} incorporation on circulating vincristine levels over 24 h after i.v. administration is shown in Fig. 2. As expected on the basis of data in Fig. 1, incorporation of G_{M1} into DSPC/Chol pH_i 4.0 liposomes containing vincristine resulted in approximately a 3-fold increase in the level of drug remaining in the plasma at 24 h (Fig. 2A). The circulating drug:lipid ratios for these systems are shown in Fig. 2B, which shows that drug release from liposomes in the plasma compartment was not influenced by incorporation of G_{M1}. For these liposomal vincristine formulations, where drug was encapsulated using the pH-gradient loading procedure in liposomes prepared in 300 mM citrate buffer at pH 4.0, greater than 90% of the encapsulated drug was released from circulating liposomes over the 24-h time course.

Retention of vincristine entrapped in 100 nm DSPC/Chol liposomes in response to a transmembrane pH gradient can be improved by decreasing the pH of the encapsulated citrate buffer from 4.0 to 2.0 (11). As shown in Fig. 2A, decreasing the pH_i from 4.0 to 2.0 resulted in a 2.5-fold increase in the circulating vincristine levels achieved at 24 h post i.v. injection. The change in intravesicular pH did not influence the clearance behavior of injected liposomes (data not shown); thus, the increased drug levels result from improved drug retention. At every time point,

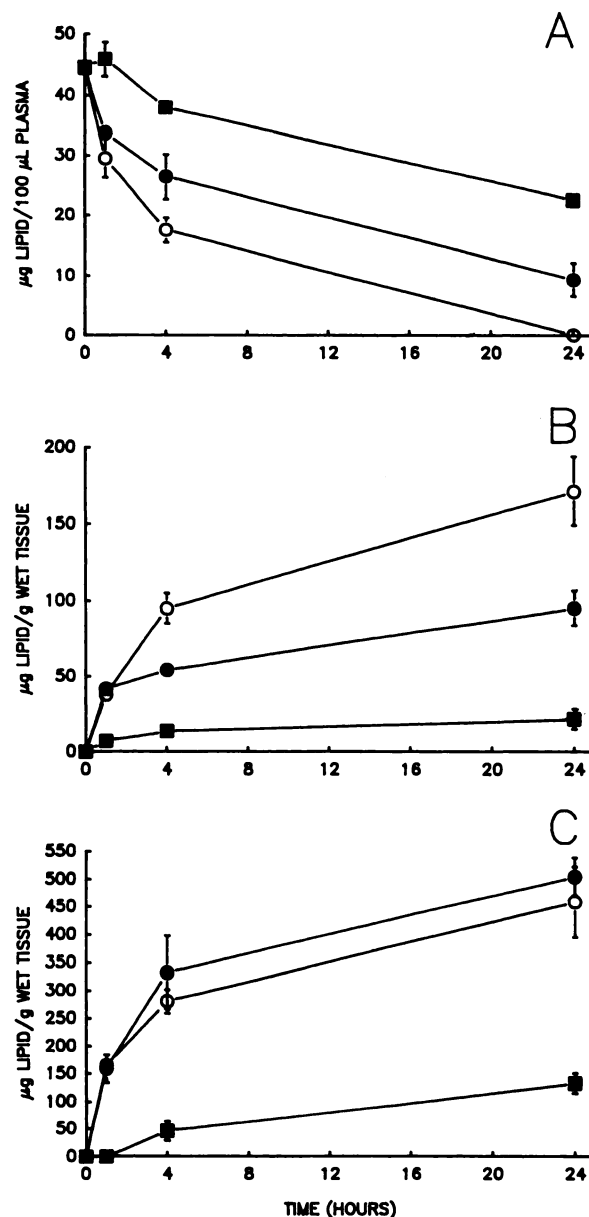


Fig. 1. Influence of G_{M1} incorporation and entrapped vincristine on the plasma clearance (A) and liver (B) and spleen (C) uptake of 100 nm DSPC/Chol liposomes. Liposomes were all administered i.v. at a lipid dose of 20 mg/kg. Vincristine was encapsulated at a drug:lipid ratio of 0.1:1 (w/w) using the transmembrane pH gradient loading technique with liposomes prepared in 300 mM citrate buffer pH_i 4.0. When present, G_{M1} was incorporated at a level of 10 mol% in DSPC/Chol liposomes (45 mol% cholesterol). Lipid levels were determined for drug free DSPC/Chol liposomes (○), DSPC/Chol liposomal vincristine (●), and DSPC/Chol/G_{M1} liposomal vincristine (■). Bars, SD from the mean using at least four mice.

higher drug:lipid ratios were observed for vincristine encapsulated in liposomes prepared at pH_i 2.0 (Fig. 2B).

Incorporation of G_{M1} in combination with the use of an interior pH_i of 2.0 resulted in an unexpected and dramatic improvement in the vincristine circulation lifetime. As shown in Fig. 2A, plasma vincristine levels were approximately 7.5- and 20- fold higher at 24 h than were achieved with comparable systems prepared in the absence of G_{M1} using the pH_i 2.0 and pH_i 4.0 buffer, respectively. As shown in Fig. 2B, DSPC/Chol/G_{M1} liposomes prepared at pH_i 2.0 exhibited less than a 20% decrease in drug:lipid ratio over 24 h in the circulation and exhibited a 12-fold increased circulation half-life as compared to DSPC/Chol pH_i 4.0 liposomes.

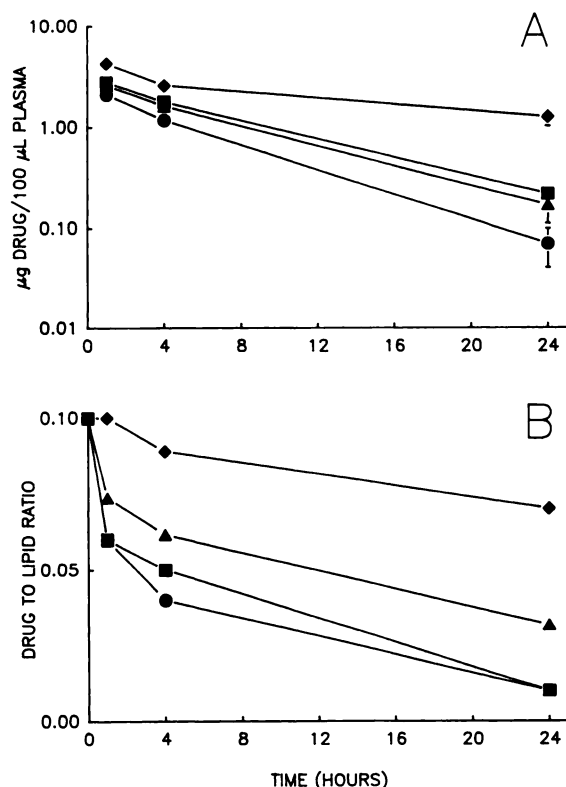


Fig. 2. Plasma clearance of vincristine (A) and associated drug:lipid ratios (B) were determined following i.v. administration in mice of liposomal vincristine prepared using DSPC/Chol pH 4.0 (●), DSPC/Chol/G_{M1} pH 4.0 (■), DSPC/Chol pH 2.0 (▲) and DSPC/Chol/G_{M1} pH 2.0 (◆) liposomes. Vincristine was encapsulated at a drug:lipid ratio of 0.1:1 (w/w) and was measured using [³H]vincristine as a tracer. Bars, SD obtained using at least four mice.

Antitumor Activity of Liposomal Vincristine. Antitumor efficacy studies were conducted to determine whether the improved vincristine circulation lifetimes achieved through the use of G_{M1} and reduced internal pH values improves the therapeutic activity of the formulation. The murine P388 antitumor activity of the DSPC/Chol/G_{M1} pH_i 2.0 liposomal vincristine preparation was compared to free drug, DSPC/Chol pH_i 4.0, DSPC/Chol pH_i 2.0, and DSPC/Chol/G_{M1} pH_i 4.0 liposomal vincristine (Table 1). All liposomal formulations of vincristine were significantly more efficacious in the P388 tumor model when compared to free drug, consistent with previous results (9). The liposomal formulation which combined the use of G_{M1} and the reduced internal pH exhibited remarkable activity. This formulation, when administered at 2, 3 and 4 mg/kg, produced long-term survival rates in excess of 50%. No long-term survivors (>70 days) were obtained with either free drug or the DSPC/Chol pH_i 4.0 liposomal vincristine formulations. Long-term survivors were seen with the DSPC/Chol preparation at pH_i 2.0 and the DSPC/Chol/G_{M1} pH_i 4.0 preparation at 4 mg/kg. Both these formulations, however, had significantly lower median survival times than the DSPC/Chol/G_{M1} pH_i 2.0 preparation. Drug-induced weight loss data (% decrease in weight on day 7) shown in Table 1 also suggests a decrease in drug toxicity for the DSPC/Chol/G_{M1} pH_i 2.0 liposomes compared to DSPC/Chol pH_i 4.0 liposomes. This preparation displayed significantly reduced weight loss at 4 mg/kg compared to the DSPC/Chol pH_i 4.0 formulation.

Discussion

Incorporation of lipids such as G_{M1} and PEG-derivatized phospholipids into liposomes can increase liposome circulation lifetime and therapeutic activity of certain liposomal drugs (12–19, 24–26). This

approach has been used for liposomal formulations of doxorubicin and cytosine arabinoside (12, 25). However, in order for G_{M1} or PEG-PE to improve the drug circulation life-time, the entrapped drug must be retained inside liposomes which reside in the plasma compartment. For example, incorporation of G_{M1} in previous liposomal vincristine preparations was of questionable value due to the relatively rapid release of drug from liposomes in the circulation (6). The advent of procedures which enhance vincristine retention (11), however, have now made the use of lipids which result in extended liposome circulation lifetimes of interest.

A decrease in the pH of the entrapped citrate buffer for DSPC/Chol liposomes leads to improved vincristine retention in the circulation as evidenced by the 3-fold increase in the circulating drug:lipid ratio of DSPC/Chol pH_i 2.0 compared to DSPC/Chol pH_i 4.0 preparations 24 h after i.v. administration (Fig. 2B). These results, when combined with the fact that including G_{M1} into DSPC/Chol liposomes at pH 4.0 results in a 2.5-fold increase in circulating liposomal lipid levels at 24 h, could suggest that plasma vincristine concentrations for G_{M1} pH_i 2.0 liposomes should be approximately 7.5-fold greater than observed for the DSPC/Chol pH_i 4.0 systems studied previously (6, 9). The data presented here indicate, however, that lowering the pH of the entrapped buffer to 2.0 and including G_{M1} in the membrane synergistically combine to dramatically increase the vincristine levels in the plasma. Specifically, 24 h after i.v. administration, circulating drug levels are increased nearly 20-fold when DSPC/Chol/G_{M1} pH_i 2.0 liposomes are used, as compared to DSPC/Chol pH_i 4.0 systems.

The mechanism whereby the use of G_{M1} and pH 2.0 entrapped citrate synergistically stabilize liposomal vincristine preparations is not understood in detail. It is of interest to note that the incorporation of G_{M1} results in no increase in drug retention for an internal pH of 4.0. However, at pH_i 2.0, there is a substantial increase in drug retention when G_{M1} is added. This is likely due to the fact that lowering the pH_i and adding G_{M1} act via different mechanisms to increase drug retention. It has been shown previously that vincristine is released from liposomes as the pH gradient across the liposome membrane decays (11). By increasing the initial pH gradient across the membrane, the drug can be retained by the liposome more effectively. The mechanism whereby incorporation of G_{M1} could decrease drug leakage is less obvious; however, it has been shown that the presence of G_{M1} decreases the amount of plasma protein binding to the liposome (26). Such a decrease in plasma protein binding can render liposome membranes less leaky (27). However, at pH_i 4.0, drug leakage may be sufficiently rapid that any stabilizing effects of G_{M1} do not significantly influence drug retention *in vivo*. The enhanced circulation lifetimes observed for the G_{M1} pH_i 2.0 liposomal vincristine preparations translate into substantially improved antitumor activity. This is consistent with previous reports that the antitumor activity of the DSPC/Chol pH_i 4.0 formulation of vincristine is significantly higher than observed for free drug or drug encapsulated in leakier liposomes containing egg yolk phosphatidylcholine. The results obtained here extend this initial observation and show that a drug with minimal activity against the murine P388 tumor model can be transformed into one where cures are achieved by improving *in vivo* drug retention properties. This is particularly evident at drug dosages above 2 mg/kg, where long-term survival rates are observed which are greater than 50%.

It is important to emphasize that the improved therapeutic activity observed here is obtained following a single i.v. dose of encapsulated vincristine. It could be argued that similar results would be achieved with a systemic infusion of free drug. Results from this laboratory (28) and others (12), however, suggest that increased therapeutic activity achieved with liposomal anticancer drugs is due primarily to accumulation of drug-loaded liposomes in the region of tumor cell growth. Furthermore,

Table 1 P388 Antitumor activity of free and liposomal vincristine in BDF₁ mice

Sample	Drug dose (mg/kg)	Lipid dose (mg/kg)	% wt change on day 7	60-day survival	Median survival (days)	% ILS ^a	L/F ^b
Saline control			+14.4	0/15	10.0		
Free vincristine	1.0		+1.9	0/5	14.0	40	
	2.0		+6.0	0/10	14.0	40	
	3.0		-3.6	0/10	12.0	20	
	4.0		-29.8	0/5	8.5	-15	
DSPC/Chol, pH 4.0; lipovinc	1.0	10	+2.8	0/5	22.0	120	1.57
	2.0	20	-2.1	0/10	27.0	170	1.93
	3.0	30	-12.0	0/10	31.0	210	2.58
	4.0	40	-24.9	0/10	32.0	220	3.76
DSPC/Chol, pH 2.0; lipovinc	2.0	20	0.0	2/5	31.0	210	2.21
	3.0	30	-10.2	2/5	36.0	260	3.00
	4.0	40	-19.0	5/5	>60.0	ND ^c	ND
DSPC/Chol/G _{M1} , pH 4.0; lipovinc	2.0	20	-4.1	0/5	21.0	110	1.50
	3.0	30	-8.4	0/5	24.0	140	2.00
	4.0	40	-22.8	1/5	24.0	140	2.82
DSPC/Chol/G _{M1} , pH 2.0; lipovinc	1.0	10	+3.3	1/5	20.0	100	1.43
	2.0	20	+0.2	8/10	>70.0	ND	ND
	3.0	30	-10.9	10/10	>70.0	ND	ND
	4.0	40	-14.4	10/10	>70.0	ND	ND

^a Percentage ILS (increase in life span) values were determined from median survival times of treated and saline control groups. If more than 50% of the animals survived for more than 70 days, median survival times and % ILS are indicated as greater than 70 days.

^b L/F (liposomal/free) values were calculated by dividing the median survival time for the liposomal vincristine (lipovinc) group by the median survival time for the group receiving the equivalent dosage of free drug.

^c ND, not determined; Chol, cholesterol.

recent pharmacological studies⁴ indicate that systemic exposure to free vincristine is lower for drug administered in liposomal form compared to untrapped vincristine. Therefore, improved drug retention characteristics should result in improved specificity of drug delivery to the tumor and hence, increased therapeutic activity. Comparable drug delivery to a defined region of disease growth would not be expected using a continuous i.v. infusion of free vincristine.

In summary, the results presented here demonstrate that low pH_i values and incorporation of G_{M1} into liposomal membranes results in a synergistic increase in vincristine retention in liposomes *in vivo*, resulting in turn in a dramatic improvement in the long-term survival rate in a P388 murine leukemia model. It is likely that these procedures will prove useful to increase drug retention of other drugs which can be loaded in response to transmembrane pH gradients to improve the therapeutic profile.

References

- Carter, S. K., and Livingston, R. B. Plant products in cancer chemotherapy. *Cancer Treat. Rep.*, 60: 1141-1156, 1976.
- Sieber, S. M., Mead, J. A. R., and Adamson, R. H. Pharmacology of antitumor agents from higher plants. *Cancer Treat. Rep.*, 60: 1127-1139, 1976.
- Owells, R. J., Hartke, C. A., Dickerson, R. M., and Hains, F. O. Inhibition of tubulin-microtubule polymerization by drugs of the *Vinca* alkaloid class. *Cancer Res.*, 36: 1499-1502, 1976.
- Owells, R. J., Owens, A. H., Jr., and Donigian, D. W. The binding of vincristine, vinblastine, and colchicine to tubulin. *Biochem. Biophys. Res. Commun.*, 47: 685-691, 1972.
- Jackson, D. V., Jr., and Bender, R. A. Cytotoxic thresholds of vincristine in a murine and a human leukemia cell line *in vitro*. *Cancer Res.*, 39: 4346-4349, 1979.
- Mayer, L. D., Nayar, R., Thies, R. L., Boman, N. L., Cullis, P. R., and Bally, M. B. Identification of vesicle properties which enhance the antitumor activity of liposomal vincristine against murine L1210 leukemia. *Cancer Chemother. Pharmacol.*, 33: 17-24, 1993.
- Jackson, D. V., White, D. R., Spurr, C. L., Hire, E. A., Pavy, M. D., Robertson, M., Legos, H. C., and McMahan, R. A. Moderate-dose vincristine infusion in refractory breast cancer. *Am. J. Clin. Oncol.*, 9: 376-378, 1986.
- Jackson, D. V., Jr., Jobson, V. W., Homesley, H. D., Welander, C., Hire, E. A., Pavy, M. D., Votaw, M. L., Richards, F., II, and Muss, H. B. Vincristine infusion in refractory gynecologic malignancies. *Gynecol. Oncol.*, 25: 212-216, 1986.
- Mayer, L. D., Bally, M. B., Loughrey, H., Masin, D., and Cullis, P. R. Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 and P388 tumors. *Cancer Res.*, 50: 575-579, 1990.
- Vaage, J., Donovan, D., Mayhew, E., Uster P., and Woodle, M. Therapy of mouse mammary carcinomas with vincristine and doxorubicin encapsulated in sterically stabilized liposomes. *Int. J. Cancer*, 54: 959-964, 1993.
- Boman, N. L., Mayer, L. D., and Cullis, P. R. Optimization of the retention properties of vincristine in liposomal systems. *Biochim. Biophys. Acta*, 1152: 253-258, 1993.
- Gabizon, A., and Papahadjopoulos, D. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. *Proc. Natl. Acad. Sci. USA*, 85: 6949-6953, 1988.
- Klibanov, A. L., Maruyama, K., Beckerleg, A. M., Torchilin, V. P., and Huang, L. Activity of amphipathic poly(ethylene glycol) 5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target. *Biochim. Biophys. Acta*, 1062: 142-148, 1991.
- Allen, T. M., Hansen, C., and Rutledge, J. Liposomes with prolonged circulation times: factors affecting uptake by reticuloendothelial and other tissues. *Biochim. Biophys. Acta*, 981: 27-35, 1989.
- Senior, J., Delgado, C., Fisher, D., Tilcock, C., and Gregoriadis, G. Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly(ethylene glycol)-coated vesicles. *Biochim. Biophys. Acta*, 1062: 77-82, 1991.
- Liu, D., and Huang, L. pH-sensitive, plasma-stable liposomes with relatively prolonged residence in circulation. *Biochim. Biophys. Acta*, 1022: 348-354, 1990.
- Klibanov, A. L., Maruyama, K., Torchilin, V. P., and Huang, L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett.*, 268: 235-237, 1990.
- Allen, T. M., and Chonn, A. Large unilamellar liposomes with low uptake into the reticuloendothelial system. *FEBS Lett.*, 223: 42-46, 1987.
- Allen, T. M., Hansen, C., Martin, F., Redemann, C., and Yau-Young, A. Liposomes containing synthetic lipid derivative of poly(ethylene glycol) show prolonged circulation half-lives *in vivo*. *Biochim. Biophys. Acta*, 1066: 29-36, 1991.
- Derksen, J. T. P., Morselt, H. W. M., and Scherphof, G. L. Processing of different liposome markers after *in vitro* uptake of immunoglobulin-coated liposomes by rat liver macrophages. *Biochim. Biophys. Acta*, 931: 33-40, 1987.
- Olson, F., Hunt, C. A., Szoka, F. C., Vail, W. J., and Papahadjopoulos, D. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. *Biochim. Biophys. Acta*, 557: 9-23, 1979.
- Bally, M. B., Nayar, R., Masin, D., Hope, M. J., Cullis, P. R., and Mayer, L. D. Liposomes with entrapped doxorubicin exhibit extended blood residence times. *Biochim. Biophys. Acta*, 1023: 133-139, 1990.
- Parr, M. J., Bally, M. B., and Cullis, P. R. The presence of G_{M1} in liposomes with entrapped doxorubicin does not prevent RES blockade. *Biochim. Biophys. Acta*, 1168: 249-252, 1993.
- Allen, T. M., Austin, G. A., Chonn, A., Lin, L., and Lee, K. C. Uptake of liposomes by cultured mouse bone marrow macrophages: influence of liposome composition and size. *Biochim. Biophys. Acta*, 1061: 56-64, 1991.
- Allen, T. M., Mehna, T., Hansen, C., and Chin, Y. C. Stealth liposomes: an improved sustained release system of 1-β-D-arabinofuranosylcytosine. *Cancer Res.*, 52: 2431-2439, 1992.
- Chonn, A., Semple, S. C., and Cullis, P. R. Association of blood proteins with large unilamellar liposomes *in vivo*: relation to circulation lifetimes. *J. Biol. Chem.*, 267: 18759-18765, 1992.
- Allen, T. M., Ryan, J. L., and Papahadjopoulos, D. Gangliosides reduce leakage of aqueous-space markers from liposomes in the presence of human plasma. *Biochim. Biophys. Acta*, 818: 205-210, 1985.
- Mayer, L. D., Bally, M. B., Cullis, P. R., Wilson, S. L., and Emerman, J. T. Comparison of free and liposome encapsulated doxorubicin tumor drug uptake and antitumor efficacy in the SC115 murine mammary tumor. *Cancer Lett.*, 53: 183-190, 1990.

⁴ L. D. Mayer, unpublished observations.

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