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ENCAPSULATION OF VINCRISTINE IN LIPOSOMES REDUCES ITS TOXICITY AND IMPROVES ITS ANTI-TUMOR EFFICACY

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ABSTRACT

Vincristine is a potent therapeutic agent with activity against a variety of tumor types. It is a cell-cycle specific agent which has exhibited enhanced anti-tumor activity when delivered in liposomal form. Vincristine can be encapsulated into large unilamellar vesicles in response to a transmembrane pH gradient with trapping efficiencies approaching 100%. The extent of vincristine encapsulation, and the subsequent retention of the drug within the liposomes, both *in vitro* and *in vivo*, are strongly dependent on the lipid composition of the liposome and on the magnitude of the transmembrane pH gradient. Liposomal formulations of vincristine have been optimized for both liposome circulation longevity, drug retention characteristics and *in vivo* anti-tumor activity. When compared to free vincristine, these formulations significantly increase the levels of vincristine remaining in the plasma after i.v. administration. These formulations also significantly increase the delivery of vincristine to tumor sites. As a consequence of the improved accumulation of vincristine at tumor sites, liposomal formulations of vincristine exhibit dramatically improved efficacy against a variety of ascitic and solid murine and human tumors than does free vincristine. Liposomal vincristine is expected to be of wide utility in a variety of human malignancies.

INTRODUCTION

The encapsulation of several antineoplastic drugs within liposomes has effected significant decreases in drug-induced toxic side effects with maintained or increased anti-tumor activity (1-12). That is, liposomal encapsulation increases the therapeutic index of some

- Indications: lymphomas
  lung cancer
  leukemia
  breast cancer
- Side Effects: peripheral neuropathy
  alopecia
  leukopenia

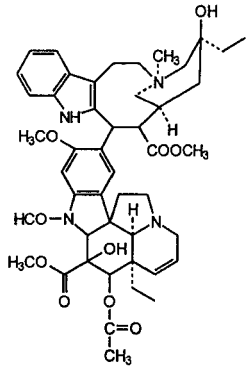

FIG 1. **Structure of the vincristine molecule and some of its biological activities.**

anticancer agents. While the majority of the previous studies have focused on doxorubicin, similar results have also been observed for vincristine (13). Vincristine is an alkaloid derived from the periwinkle plant that possesses anti-tumor activity against a wide range of neoplasms including both the Hodgkin's and non-Hodgkin's lymphomas, acute lymphoblastic leukemia, embryonal rhabdomyosarcoma, neuroblastoma, breast carcinoma, and Wilm's tumor (14,15). The chemical structure and some of the biological effects of vincristine are shown in Figure 1. Vincristine exerts its cytotoxic activity by arresting cell growth exclusively during metaphase by attaching to the growing end of microtubules and preventing their assembly (16,17).

Since the vinca alkaloids are cell-cycle-specific cytotoxic drugs, it may be predicted that increased ability to maintain high blood levels of drug for extended periods would be therapeutically advantageous. Previous studies with doxorubicin have shown that drug encapsulation in liposomes leads to increased drug circulation time (18) and increased anti-tumor activity (19). Consequently, it can be hypothesized that the therapeutic activity of a cell-cycle-specific drug such as vincristine would be particularly sensitive to the circulation lifetimes compared to drugs such as doxorubucin that possess activity independent of specific points in the cell cycle. This relationship has been demonstrated *in vitro* by Jackson and Bender (20), and has been confirmed in our laboratory using the L1210 leukemic cell line (Table 1). Specifically, increasing the exposure of L1210 cells *in vitro* to vincristine from 1 to 72 h, results in a 10^5-fold decrease in the concentration of drug needed to yield 50% cytotoxicity (IC_{50}^1). In contrast, when

TABLE I

Effect of the total duration of exposure to either vincristine or doxorubicin on the cytotoxicity of L1210 cells *in vitro*. Inhibitory concentration required for 50% cytotoxicity (IC_{50}) data for vincristine were obtained from (26); data for doxorubicin from Masin and Nayar (unpublished).

	IC_{50} (nM)	
Exposure Time (h)	Vincristine	Doxorubicin
1	12000	370
6	2400	55
24	7.3	18
72	0.12	9.2
1h IC_{50}/72h IC_{50}	10^5	40

the exposure time for doxorubicin was increased from 1 to 72 h, there was only a 40-fold decrease in the IC_{50}. If liposomal encapsulation and delivery results in higher levels of drug at tumor sites for longer periods of time, greatly improved efficacy may be expected.

This chapter will examine the effect of various parameters on improving vincristine circulation longevity and tumor exposure by liposomal encapsulation with the aim of reducing toxicity and improving the anti-tumor activity of the drug.

DRUG LOADING OF VINCRISTINE INTO LIPOSOMES

It is well established that the distribution of weak bases and acids across biological membranes is strongly influenced by the presence of transmembrane pH-gradients (21,22). Previous work has demonstrated that in the presence of a pH-gradient (inside acidic), doxorubicin will rapidly accumulate into both egg phosphatidylcholine (EPC) and EPC/cholesterol (1/1) LUVs (23).

Many biological compounds or pharmaceutical agents (such as doxorubicin and vincristine) are weak bases. The dissociation constant (K_a) for a weak base can be described as follows:

$$K_a = [H^+][D]/[DH^+] \quad (1)$$

where $[H^+]$ is the hydrogen ion concentration, [D] is the concentration of the neutral form of the weak base and $[DH^+]$ is the concentration of the protonated drug. The pK_a is the negative log of K_a. According to the Henderson-Hasselbach equation:

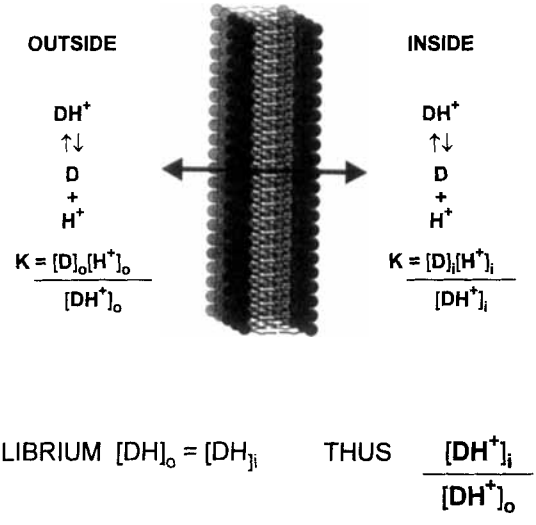

FIG 2. Redistribution of weak bases in response to transmembrane pH gradients; where D is the drug/weak base of interest.

$$pH = pK_a + \log([D]/[DH^+]) \quad (2)$$

Assuming that K_a is the same on both sides of the liposomal membrane, it follows that:

$$K_a = [H^+]_i[D]_i/[DH^+]_i = [H^+]_o[D]_o/[DH^+]_o \quad (3)$$

where the subscripts $_i$ and $_o$ refer to the inside and outside of the liposome. Since the neutral form of ionizable molecules are usually orders of magnitude more membrane permeable than the charged species (24,25), at equilibrium the concentration of the neutral species will be the same on both sides of the membrane. Therefore, if $pK_a >> pH_o >> pH_i$:

$$[DH^+]_i/[DH^+]_o = [H^+]_i/[H^+]_o \quad (4)$$

In the case of a drug such as vincristine that contains two basic functions ($pK_1 = 5.0$ and $pK_2 = 7.4$ with dissociation constants K_{d1} and K_{d2}, respectively), it can be shown, in the absence of membrane partitioning effects and assuming that $[H^+]_o$, $[H^+]_i >> K_{d1}$, K_{d2}, that:

$$[D]_i/[D]_o = [H^+]_i^2/[H^+]_o^2 \quad (5)$$

and thus the rate of drug release increases proportional to the inverse square of the internal proton concentration (see Fig. 2).

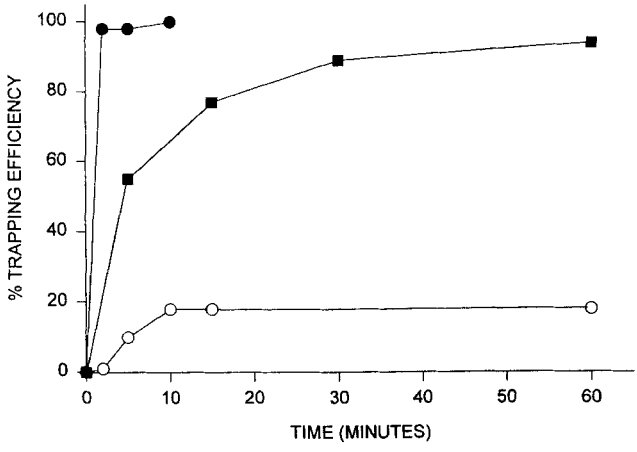

FIG 3. Uptake of vincristine into DSPC/cholesterol (●,○) and EPC/cholesterol (■) vesicles at 21oC (○,■) and 60oC (●). Data from (13).

IN VITRO LOADING AND RETENTION OF LIPOSOMAL VINCRISTINE

Initial studies examined the encapsulation of vincristine inside EPC/cholesterol (55/45; mol/mol) and distearoylphosphatidylcholine (DSPC)/cholesterol (55/45; mol/mol) vesicles using pH-gradient (inside acidic) driven drug uptake processes (13). With an internal pH of 4.0 and an external pH of 7.5, trapping efficiencies in excess of 98% can be achieved using drug-to-lipid ratios as high as 0.2/1 (wt/wt). Vincristine rapidly accumulates inside EPC/cholesterol vesicles across a pH-gradient at 21°C, leading to >90% drug entrapment within 30 minutes. Drug entrapment within DSPC/cholesterol vesicles is slower at room temperature with only 17% drug entrapment achieved over 1 h at 21°C (Fig. 3). However, when the temperature was increased to 60°C, >98% of the vincristine was entrapped within 10 minutes of incubation (Fig. 3).

Vincristine retention within both liposome formulations is dependent on the ability of each system to maintain a large pH-gradient (13). DSPC/cholesterol liposomes do not release detectable levels of vincristine over 24 h at 21°C (Fig. 4A). EPC/cholesterol vesicles, however, released approximately 40% of entrapped drug after 24 h under identical conditions (Fig. 4A). Similarly, the rate of collapse of the transmembrane pH-gradient was comparable to the rate of vincristine release from both EPC/cholesterol and DSPC/cholesterol vesicles (compare Fig. 4A and Fig. 4B). Similar results have been obtained for the incubation of liposomes in the presence

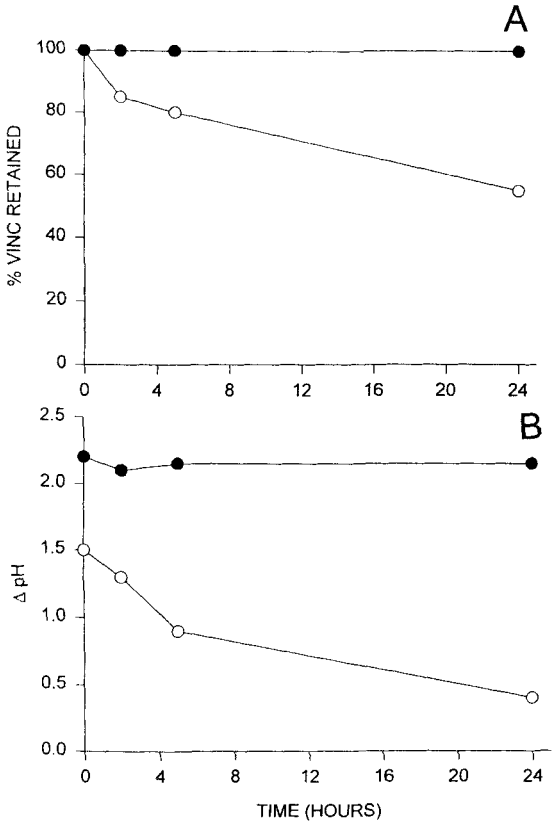

FIG 4. (A) vincristine release from DSPC/cholesterol (●) and EPC/cholesterol (○) vesicles subsequent to pH-dependent drug accumulation. Vincristine was encapsulated at a drug/lipid ratio (wt/wt) of 0.19 (± 0.01). Liposomes were incubated at 21oC. (B) transmembrane pH gradient dissipation at 21oC in DSPC/cholesterol (●) and EPC/cholesterol (○) vesicles subsequent to vincristine encapsulation at a drug/lipid ratio (wt/wt) of 0.19 (± 0.01). Incubation conditions were identical to those described in (A). Data from (13).

of whole blood at 37°C (26). EPC/cholesterol vesicles with an initial interior pH of 4.0 (initial $pH_i = 4.0$) rapidly released vincristine upon *in vitro* exposure to blood, resulting in only 4% drug retention after 24 h of incubation. This rapid release of drug was accompanied by a parallel reduction in the magnitude of the pH-gradient across the vesicle membrane. In contrast, DSPC/cholesterol vesicles retained approximately 20% of entrapped drug after 24 h under the same conditions and exhibited an increase in the stability of the pH-gradient, as compared to the EPC/cholesterol system (26).

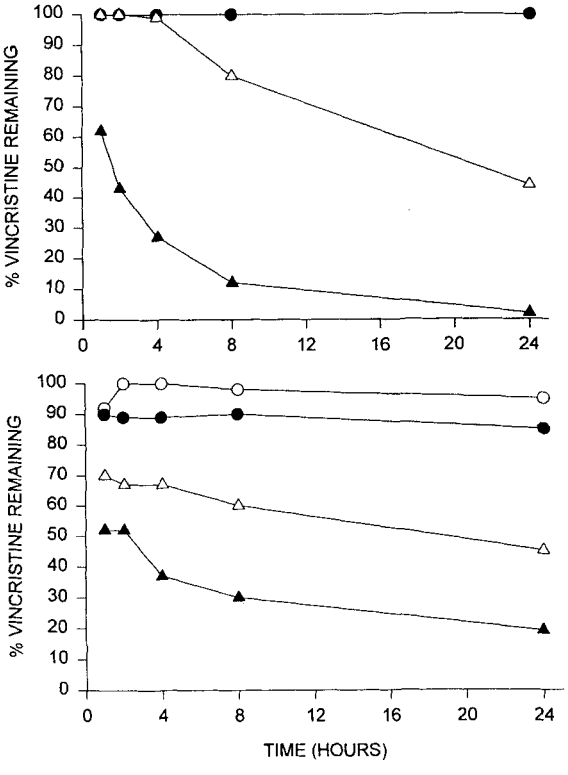

FIG 5. Vincristine release from 100-nm DSPC/cholesterol vesicles incubated in buffer (A) and mouse serum (B) at 37oC for internal pH of 2.0 (○), 3.0 (●), 4.0 (Δ), and 5.0 (▲). Internal buffering capacity was 300 mM citrate for all systems. Initial drug-to-lipid ratios were 0.1/1 (wt/wt). Data from (27).

A model based on the assumption that only the neutral form of the vincristine molecule is membrane permeable (24,25) predicts that the rate constant for transbilayer movement of vincristine should be proportional to the inverse square of the intraliposomal proton concentration. Therefore, if the interior pH of the liposomes is lowered, the retention of encapsulated vincristine should be significantly greater. In experiments with DSPC/cholesterol LUVs having interior pH values of 2.0, 3.0, 4.0, and 5.0 (employing a 300 mM citrate buffer) (27). With initial intraliposomal pH values of 3.0 or lower, 100% retention of vincristine over 24h in the presence of both buffer and mouse serum was observed. Increased leakage of vincristine, in the presence of either buffer or mouse serum, corresponded with higher intraliposomal pH values (Fig. 5).

The effect of lower intraliposomal pH values on increasing vincristine retention in liposomal systems is mediated by two factors. The first of these is the improved internal buffering capacity that is a consequence of the initial interior pH values that are below the lowest pK (pK_1 = 3.13) of the carboxyl functions of the citrate buffer. However, the second and more important factor is the dependence of the rate constant for vincristine movement across the lipid bilayer on the inverse square of the proton concentration. This relationship predicts a 100-fold reduction in vincristine leakage rates for every unit the interior pH is lowered.

In conclusion, the internal pH is an extremely important parameter enhancing liposomal vincristine loading and retention *in vitro*. The retention of vincristine in liposomes is improved in those liposomes exhibiting reduced permeability properties and stable transmembrane pH-gradients following drug uptake. Further increases in drug retention can be achieved by lowering the internal pH of the vesicles. The effects of these factors on the *in vivo* activity of liposomal vincristine is discussed below.

BIOLOGICAL PROPERTIES OF LIPOSOMAL VINCRISTINE

Toxicology

One of the primary goals of the encapsulation of cytotoxic agents in liposomes is to ameliorate the toxic side effects associated with these compounds. The major side effects arising from the administration of vincristine are peripheral neuropathy, alopecia, leukopenia, as well as soft tissue necrosis and ulceration. As demonstrated for liposomal doxorubicin (4), encapsulation of vincristine in liposomes can reduce the toxicity associated with either i.v. or s.c. administration. Specifically, encapsulation of vincristine in DSPC/cholesterol liposomes results in a 1.7 to 2.1-fold increase in the LD_{50} values compared to the free drug when administered i.v. to CD-1 mice (Table 2) (13). Encapsulation of vincristine also resulted in decreased weight loss following i.v. administration of liposomal formulations having good drug retention characteristics (13,28,29).

A comprehensive comparison of the toxicity of free and liposomal vincristine after single and multiple i.v. injections concluded that liposomal vincristine was less toxic than was the free

TABLE 2
Acute toxicity of free and liposomal vincristine after i.v. administration. The 50% lethal dose (LD_{50}) values were obtained from (13).

Sample	Mouse Strain	LD_{50} (mg/kg)
Free Vincristine	DBA/2J	2.5
Liposomal Vincristine	DBA/2J	4.2
Free Vincristine	CD-1	1.9
Liposomal Vincristine	CD-1	4.8

drug in mice and had very similar toxicity to the free drug in beagle dogs (30). In a recent comparison of the dermal toxicity of free and liposomal vincristine, it was demonstrated that vincristine encapsulation in liposomes significantly reduced the occurrence of skin necrosis and ulceration, and also markedly decreased the severe inflammatory response at the site of i.v. injection (31). Therefore, liposomal encapsulation can reduce the potential of vincristine to cause tissue necrosis upon accidental extravasation. This could improve the safe administration of vincristine by both standard and alternative routes. Overall, these studies clearly indicate that the encapsulation of vincristine in liposomes can reduce the cytotoxicities normally associated with this drug.

Pharmacokinetics and Tumor Loading

Liposomes can extend the circulation times and improve tumor uptake of a wide variety of drugs. One of the primary factors that influences the circulation longevity of an encapsulated agent is the retention characteristics of the liposomal carrier. For this reason, studies have focused on the effects of lipid composition on the vincristine retention within liposomes. Vincristine that was encapsulated into EPC/cholesterol liposomes using the pH-gradient method (pH_i=4.0), for example, rapidly leaked from the liposomes both *in vitro* (see above) and *in vivo* after i.v. administration in DBA/2J mice (26). Substitution of the EPC with DSPC significantly improved the retention of vincristine both *in vitro* (see above) and *in vivo* (26). Further

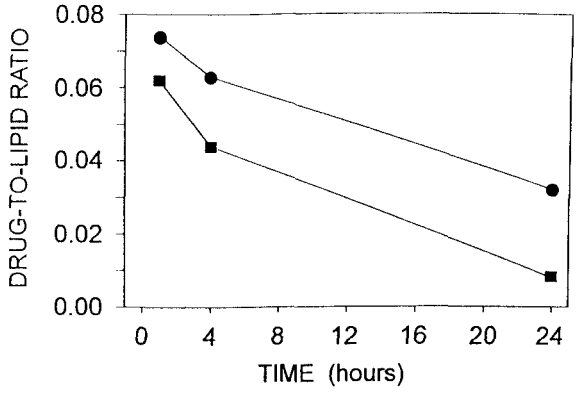

FIG 6. Influence of intraliposomal pH on vincristine retention in 120 nm LUVs of DSPC/cholesterol during in vivo circulation in BDF1 mice. Liposomes containing vincristine at an initial drug/lipid ratio of 0.1/1 (wt/wt) were administered to BDF1 mice at a dose of 2 mg vincristine/kg (20 mg lipid/kg). The drug /lipid ratio in the plasma at various times was determined as described in (27).

improvements to the *in vivo* retention of vincristine in liposomes have been achieved by the reduction of the intraliposomal pH to 2.0 (Fig. 6) (27,28), by the presence of 10 mol% of G_{M1} in DSPC/cholesterol liposomes (and pH_i=2.0) (28), and by the substitution of DSPC with sphingomyelin (29). These latter two liposomal vincristine formulations exhibit excellent drug retention properties, with 70-75% of the originally encapsulated vincristine remaining in the liposomes after 24 hours in the circulation of BDF_1 mice (28,29).

As expected, encapsulation of vincristine significantly increases the plasma vincristine levels in the circulation over those observed after i.v. administration of free vincristine. In SCID mice treated with either free vincristine or with vincristine encapsulated in either DSPC/cholesterol or sphingomyelin/cholesterol liposomes, the plasma levels of vincristine achieved with the liposomal drug were approximately 50 to 100-fold higher than achieved with free vincristine (Fig. 7) (29). When the liposomal formulations are further optimized for improved retention of encapsulated vincristine, these changes are closely associated with increased plasma vincristine levels. For example, those formulations that have improved drug

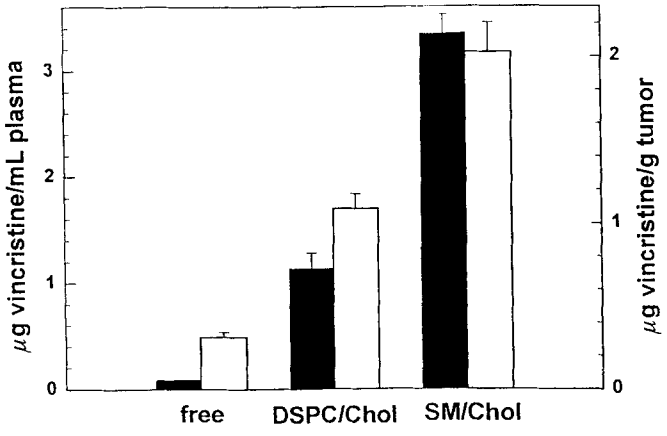

FIG 7. Plasma (filled bars) and tumor (open bars) levels of vincristine 24 hours after the administration of free and liposomal vincristine in SCID mice bearing solid human A431 tumors. SCID mice bearing two A431 tumors were injected i.v. with free vincristine (□) or with large unilamellar liposomes of DSPC/cholesterol (○) or sphingomyelin/cholesterol (●) containing vincristine at a drug/lipid ratio of 0.1 (wt/wt). Vincristine was injected at a dose of 2.0 mg/kg, representing a lipid dose of 20 mg/kg. Data from Webb, Bally and Mayer (unpublished observations).

retention characteristics as a consequence of either their lipid composition, such as DSPC *vs.* EPC (26); DSPC/cholesterol/G_{M1} at pH_i=2.0 (28); or sphingomyelin *vs.* DSPC (29) or the magnitude of the transmembrane pH gradient (i.e. pH_i=2.0 vs. 4.0) (27,28) typically have far greater amounts of vincristine remaining in the plasma after i.v. administration than do liposomal formulations that more rapidly leak the entrapped drug (Fig. 7). These observations are consistent with the findings that the vincristine that is released from liposomes *in vivo* is removed very rapidly from the circulation (St. Onge, G. and Mayer, L.D., unpublished observation) and that free vincristine administered i.v. is also removed very quickly from the circulation (Fig. 7) (26,29,32).

These considerations assume that the liposomes used for drug encapsulation have relatively similar circulation longevities. However, drug retention alone is not the sole determinant of plasma vincristine levels. For example, DSPC/cholesterol liposomes with

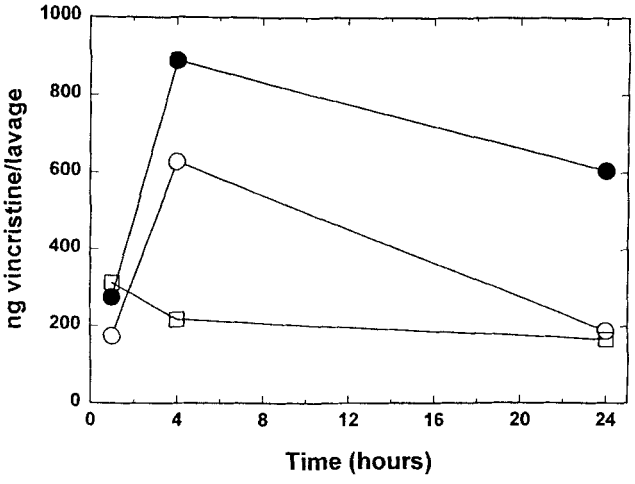

FIG 8. Accumulation of vincristine in peritoneal P388 tumors in BDF1 mice after i.v. administration of free vincristine (□) or of large unilamellar liposomes of DSPC/cholesterol (○) or sphingomyelin/cholesterol (●) containing vincristine at a drug/lipid ratio of 0.1/1 and a vincristine dose of 2.0 mg/kg.

pH_i=4.0 and a mean diameter of approximately 600 nm retain vincristine effectively, but these liposomes are rapidly removed from the circulation as a consequence of their large size and, overall, give low plasma vincristine levels following i.v. administration (26). Conversely, while the addition of 5 mol% of PEG_{2000}-DSPE to liposomes of sphingomyelin/cholesterol increased the circulation lifetime of the liposomes, compared to those lacking PEG_{2000}-DSPE, but it also increased the leakage of vincristine from these liposomes (29)

Increased plasma vincristine levels achieved by the encapsulation of vincristine in liposomes are closely associated with increased drug accumulation at the site of tumor growth. Encapsulation of vincristine in liposomes composed of DSPC/cholesterol resulted in marked increases in the amount of vincristine accumulated at the site of peritoneal ascitic L1210 (32) and P388 tumor cell inoculation in BDF_1 mice (Fig. 8) (29) compared to free vincristine. Similarly, encapsulation of vincristine in DSPC/cholesterol liposomes also resulted in significant increases in vincristine accumulation in solid B16/BL6 melanoma tumors in BDF_1 mice (32) as well as in

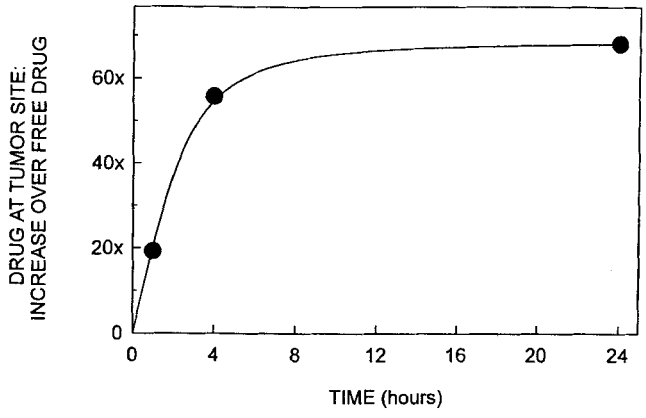

FIG 9. Increase in the accumulation of vincristine at the site of a peritoneal P388 tumor in BDF1 mice as a consequence of encapsulation in liposomes. Data represents the ratio of vincristine accumulated due to delivery in sphingomyelin/cholesterol liposomes to that delivered by free vincristine.

solid human A431 xenograft tumors in SCID mice (Fig 7) (29). Attempts have been made to quantify the improvement in vincristine accumulation at tumor sites due to encapsulation in liposomes. Consistent with the observation of 50 to 100-fold increases in plasma vincristine levels as a consequence of drug encapsulation in liposomes (Fig. 7) (29), treatment with sphingomyelin/cholesterol formulations of vincristine resulted in a 60-fold increase over free vincristine in the amount of drug accumulated at peritoneal P388 tumors in BDF_1 mice (Fig. 9). At 72 hours after administration, the trapezoidal area under the curve (AUC) values were 12.9-(L1210 tumors) and 4.1-fold (B16/BL6 tumors) greater for the liposomal vincristine compared to the free vincristine (32). Liposomal formulations of vincristine that were further optimized for improved drug retention characteristics (sphingomyelin/cholesterol liposomes) have increased plasma vincristine levels that are closely correlated with increased vincristine accumulation at the site of peritoneal P388 tumors in BDF_1 mice as well as in solid human A431 xenograft tumors in SCID mice (Fig. 7) (29).

Previous work with liposomal doxorubicin (33) has shown, by analysis of the pharmacology of both drug and lipid, that drug accumulation in tumors is due to the

extravasation of the liposome and its encapsulated drug. Similarly, the accumulation of vincristine in both L1210 and B16/BL6 tumors was not due to the extravasation of free drug released from liposomes in the circulation. Rather, vincristine accumulation at the tumors results from extravasation of the liposomes containing the encapsulated drug (32). Since delivery of vincristine to the tumor site results primarily from the extravasation of the liposome and encapsulated drug, the importance of optimizing the retention of drug within the liposomal carriers is obvious.

Anti-tumor Efficacy of Liposomal Vincristine

A significant increase in the anti-tumor efficacy of vincristine, occurring as a consequence of drug encapsulation in liposomes, was first demonstrated with DSPC/cholesterol liposomes (pH_i=4.0). Administration of free and liposomal vincristine at drug doses in the range between 0.5 and 3.0 mg/kg resulted in significant increases in the mean survival times and in the Percent Increase in Life Span (%ILS) values of DBA/2J mice bearing either P388 or L1210 peritoneal tumors (13). The activity of vincristine against L1210 tumors in DBA/2J mice appeared to be closely correlated to the vincristine circulation longevity in the formulation (26). For example, free vincristine was rapidly removed from the circulation after i.v. administration and treatment with free vincristine resulted in a small increase in survival (%ILS = 28%). Formulations having extended vincristine circulation lifetimes (i.e. 120 nm diameter liposomes of DSPC/cholesterol) had the greatest anti-tumor efficacy (%ILS = 133) (26). Further increases in the circulation longevity of the encapsulated drug, achieved by an increase in the transmembrane pH gradient (pH_i=2.0), caused a dramatic increase in therapeutic activity (Fig. 10). The encapsulation of vincristine in DSPC/cholesterol liposomes also caused a significant increase in anti-tumor activity against solid B16/BL6 tumors in BDF_1 mice when compared to free vincristine (32). Vincristine encapsulated in DSPC/cholesterol liposomes containing 10

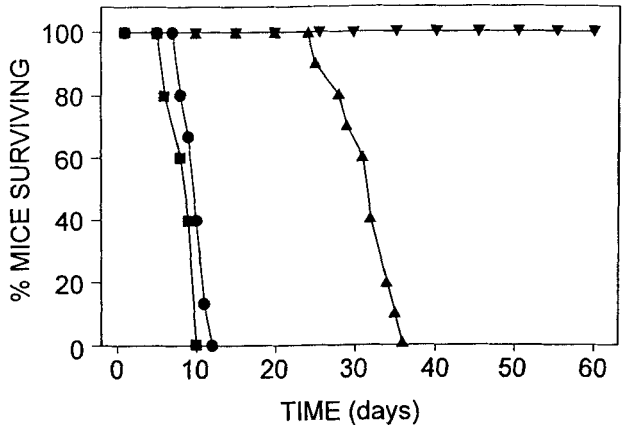

FIG 10. Influence of intraliposomal pH on the efficacy of vincristine encapsulated in DSPC/cholesterol liposomes against P388 tumors. BDF1 mice bearing peritoneal ascitic P388 tumors were untreated (■) or were treated with free vincristine (●) or large unilamellar vesicles of DSPC/cholesterol with encapsulated vincristine and intraliposomal pH of either 2.0 (▲) or 4.0 (▼). Boman, N.L. and Cullis, P.R., unpublished observations.

mol% of the ganglioside G_{M1}, a formulation of vincristine exhibiting maximal drug circulation lifetimes obtained to date, have excellent activity against P388 ascitic peritoneal tumors in BDF_1 mice (28). Specifically, at a drug dose of 3.0 mg/kg, free vincristine increased the median survival of BDF_1 mice bearing P388 tumors by only 2 days (from 10 days in saline-treated controls to 12 days). In comparison, DSPC/cholesterol/G_{M1} liposomes containing vincristine and administered at a vincristine dose of 3 mg/kg resulted in a 100% cure rate (i.e. 100% survival at 60 days) (28). Similarly, liposomes composed of sphingomyelin/cholesterol have drug retention and vincristine circulation longevity properties comparable to those obtained with G_{M1}-containing liposomes and, when administered at 3 mg vincristine/kg to BDF1 mice bearing peritoneal P388 tumors, caused a 90% cure rate at 60 days (29). Finally, a comparison of the activity of free and liposomal vincristine against solid human A431 xenograft tumors in SCID mice demonstrated a dramatic effect of the encapsulation of vincristine on anti-tumor activity (29). SCID mice bearing the A431 tumors and receiving no treatment have extensive tumor

growth and required termination after 10 days. Administration of free vincristine (2 mg/kg) causes a small delay in tumor growth but did not influence the endpoint. Treatment with vincristine encapsulated in DSPC/cholesterol liposomes causes a 10 day delay in tumor growth. Treatment with vincristine encapsulated in sphingomyelin/cholesterol liposomes results in a complete inhibition of tumor growth, with no detectable change in tumor size occurring for 40 days after treatment (29). Taken in sum, these studies clearly demonstrate that the encapsulation of vincristine in liposomes has a profound effect on the pharmacokinetics of the drug, on its accumulation in sites of both ascitic and solid tumors and, as a result, on anti-tumor efficacy.

SUMMARY

Vincristine is a potent antineoplastic agent currently in wide clinical use and possessing a range of indications and cytotoxic side-effects. As a consequence of its cell-cycle-specific activity, vincristine is an ideal candidate for improved therapeutic activity by extending its circulation longevity. As a consequence of its chemical properties, vincristine can be readily encapsulated into, and retained within, liposomes. The encapsulation of vincristine in optimized liposomal formulations profoundly improves the toxicity of the drug, the circulation longevity of the drug after i.v. administration and the accumulation of the drug at tumor sites. Liposomal formulations of vincristine have significantly improved anti-tumor activity against a variety of ascitic and solid tumors, both murine and human. Taken in sum, these studies suggest that liposomal vincristine may have wide applicability in the treatment of human malignancies.

FOOTNOTES

[1]abbreviations: area-under-the-curve, AUC; distearoylphosphatidylcholine, DSPC; egg phosphatidylcholine, EPC; inhibitory concentration required for 50% cytotoxicity, IC_{50}; lethal dose required for 50% survival, LD_{50}; large unilamellar vesicle, LUV; severe combined immuno-deficiency, SCID.

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