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# Liposome-Encapsulated Actinomycin D: Potential in Cancer Chemotherapy[1] (38268)

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Actinomycin was the first crystalline antibiotic derived from a Streptomyces (1). Brockmann and his associates first demonstrated in 1952 that actinomycin C had a remarkable effect in repressing the growth of tumors such as Hodgkin's disease and lymphomas, both in animals and in man (2). Subsequent studies throughout the world have shown that actinomycins are indeed very effective inhibitors of the growth of a variety of tumors. The excessive toxicity of various preparations of actinomycins, including actinomycin D, however, has been the most serious factor preventing the general use of these antibiotics in cancer chemotherapy (3, 4).

We have recently reported that chelating agents such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) can be encapsulated within lipid spherules, i.e., liposomes (5, 6). These chelating agents, when injected into mice in a liposome-encapsulated form, were shown to be retained longer and in higher concentrations in the mouse tissues than when injected in a nonencapsulated form (5, 6). The liposome-encapsulated form was also found to be more effective in removing various toxic metals from mouse tissues (7).

We report here the preparation of liposome-encapsulated actinomycin D, its reduced toxicity to mice, and some preliminary results on the use of liposomal actinomycin D for the treatment of mice bearing Ehrlich ascites tumor.

*Materials and Methods.* The liposomes were prepared with a mixture of 3.0 mg of egg lecithin (Sigma Chem. Co., St. Louis, MO), further purified as described previously (5, 6) and 3.0 mg of cholesterol (Applied Sciences Laboratories Inc., State College, PA) dissolved in chloroform. Actinomycin D (Merck, Sharp and Dohme, West Point, PA) dissolved in 8 m*M* $CaCl_2$ at a concentration of 0.5 mg in 1 ml, was slowly added to the dried film of lecithin–cholesterol mixture with immediate and constant stirring. The subsequent steps in liposome preparation are essentially the same as described previously (5, 6). The liposomes containing actinomycin D were finally resuspended in 8 m*M* $CaCl_2$ for injection. The amount of actinomycin D encapsulated within liposomes was determined by using $^3$H-actinomycin D (Amersham/Searle Corp., Arlington Heights, IL), and the subsequent analysis of radioactivity in the liposomes was used to calculate the doses for injection.

Female CF#1 (Carworth Farms) mice, 3 mo of age, weighing between 27 and 29 g, were used throughout these experiments. For toxicity studies, groups of 15 or 20 mice were given actinomycin D at different levels, either in the nonencapsulated or in the liposome-encapsulated form. Toxicity of actinomycin D was tested by intravenous or intraperitoneal injection.

Two experiments were carried out to test the therapeutic efficacy of encapsulated ac-

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tinomycin D against Ehrlich ascites (E.A.) tumor cells *in vivo*. In Experiment I, 30 mice received $20 \times 10^6$ E.A. cells by intraperitoneal injection. After 5 days, separate groups of 10 mice each were treated with one of the following: (1) 0.4 ml of 8 m*M* $CaCl_2$ (control), (2) nonencapsulated actinomycin D (0.75 mg/kg), or (3) liposome-encapsulated actinomycin D (0.75 mg/kg). In Experiment II, two groups of 10 mice were similarly inoculated with E.A. cells. Treatment of one group was begun after 3 days with 0.5 mg/kg of encapsulated actinomycin D, followed by injection on 4 consecutive days with 0.25 mg/kg, also encapsulated; while the control group received $CaCl_2$ at the same time intervals. All injections were given intraperitoneally, in volume of 0.4 ml in Experiment I, 0.2 ml in Experiment II.

For electron microscopic studies, a mouse was inoculated with E.A. cells in the same manner as described above. Five days after the inoculation, liposomes containing actinomycin D (at a concentration of 2 mg/kg) were injected intraperitoneally. At intervals of 1, 6, 12, and 24 hr after the liposome injection, 0.2 ml of ascitic fluid was aspirated from the mouse abdominal cavity with a syringe. The fluid was centrifuged, and the pellet of E.A. cells was fixed and processed for examination in a JEOLCO JEM-7A electron microscope by methods previously described (8).

*Results.* The $LD_{50}$ as determined by probit analysis for actinomycin D given intravenously was 0.43 ± 0.04 mg/kg. At doses of 0.5 mg/kg and above, most of the deaths occurred within 24 hr; at the lowest dose used, 0.3 mg/kg, the average survival time of the 8 decedents (out of 20 injected) was 3.5 days. After intraperitoneal injection, the $LD_{50}$ was 0.59 ± 0.17 mg/kg, with some of the decedents surviving as long as 14 days. Animals were observed for at least 24 days.

In contrast, we have observed no deaths in mice given liposome-encapsulated actinomycin D, after either intravenous (1 mg/kg) or intraperitoneal (2 mg/kg) injection. In neither case were there any obvious manifestations of drug toxicity.

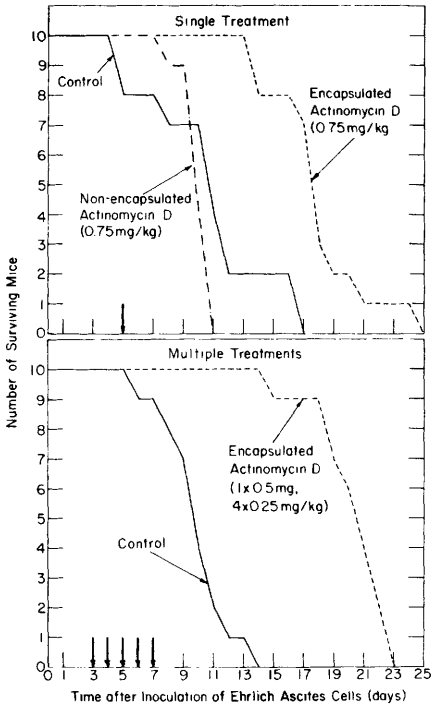


FIG. 1. Therapeutic effect of liposome-encapsulated actinomycin D on survival of mice bearing Ehrlich ascites (E.A.) tumor. (Top) Single treatment given at day 5 after inoculation of $20 \times 10^6$ E.A. cells: (1) control, (2) liposome-encapsulated actinomycin D (0.75 mg/kg), (3) nonencapsulated actinomycin D (0.75 mg/kg). (Bottom) Multiple daily treatments starting at day 3 after inoculation of $20 \times 10^6$ E.A. cells: (1) control, (2) liposome-encapsulated actinomycin D; first dose of 0.50 mg/kg, followed by four consecutive injections of 0.25 mg/kg.

The effects of treatment with actinomycin D on the survival of mice bearing E.A. tumor are presented in Fig. 1. After a single injection of encapsulated actinomycin D (0.75 mg/kg), the average survival time of mice, after treatment, increased from 7.4 ± 3.11 days in untreated control mice to 13.2 ± 3.19 days ($P = 0.001$). The survival time of mice receiving nonencapsulated actinomycin D was slightly but not significantly less than the untreated controls. After multiple injections of encapsulated actinomycin D, the average survival time was increased to 17.6 ± 2.17 days vs 7.3 ± 2.26 days in the controls ($P < 0.001$). All

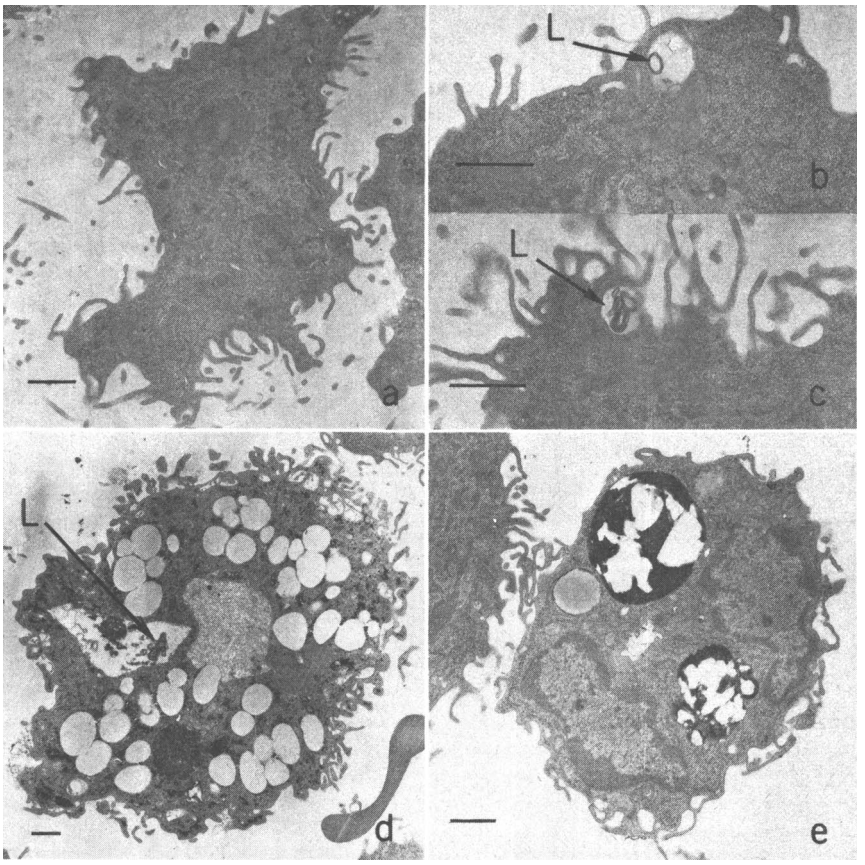


FIG. 2. Effect of liposome-encapsulated actinomycin D on the morphology of Ehrlich ascites tumor cells. (a) An Ehrlich ascites cell before treatment. (b) and (c) Liposomes (L) seen within the cytoplasm of two Ehrlich ascites cells, one hour after the injection of liposomes containing actinomycin D. (d) An Ehrlich ascites cell, 1 hr after the injection of liposomes, with numerous lipid droplets, clumps of ribosomes, and a degenerating nucleus. A liposome-like figure (L) can be seen in an autophagic vacuole. (e) An Ehrlich ascites cell, 12 hr after the injection of liposomes, with two large autophagic vacuoles.

All the cells were obtained from the same mouse, inoculated with $20 \times 10^6$ Ehrlich ascites cells; at day 5, an injection of liposomes containing 2.0 mg/kg of actinomycin D was given intraperitoneally.

of the control animals had died before the first death occurred in the treated group.[2] Nonencapsulated actinomycin D could not be used in this regime because of its toxicity.

Liposomes containing actinomycin D were found within E.A. cells one hour after the injection of liposomes (Fig. 2). Morphological alterations of endoplasmic reticulum and nuclei in the E.A. cells were also observed as early as 1 hr after the liposomal actinomycin D injection. Severe cell damage, such as striking increases in numbers of autophagic vacuoles and clumping of ribosomes, as well as dead cells was seen in samples taken at subsequent time intervals after injection.

*Discussion.* The underlying mechanism for the drastic reduction in toxicity of actinomycin D when it is encapsulated in liposomes is not fully understood. The drug

[2] In a separate experiment, we observed that injection of liposomes prepared with KCl instead of actinomycin D had no effect on survival.

is undoubtedly released slowly and gradually from the liposomes. We have not established how much of the drug may be released before or after uptake of the liposomes by the tumor cells. Actinomycin D is known for its selective cytotoxicity to bone marrow and intestinal epithelium (3, 4). It is possible that the reduction in toxicity achieved by liposome encapsulation reflects a diminished absorption of the drug by these tissues. The mechanism of release of actinomycin D from liposomes within the E.A. cells is also not clear. We are currently investigating the possible participation of lysosomal enzymes in this process of drug release.

While our present studies were in progress, Gregoriadis reported a method of preparing liposomes with actinomycin D entrapped in the lipid phase (9), which differs from our method of encapsulation inside the lipid bilayers. Our studies with $^{3}$H-labeled actinomycin D showed only 2.4% of the injected radioactivity in the circulation 1 hr after injection, whereas Gregoriadis found that 38% of their injected radioactivity was still present in the circulation 2.5 hr after injection. No biological studies were reported by this author; therefore, no further comparison is possible. We have also successfully prepared liposomes containing other antitumor agents, but the biological activities of these liposomes are still to be investigated.

We have presented here the following observations: (1) Actinomycin D, when encapsulated within liposomes, is much less toxic to mice than the nonencapsulated form. (2) Liposome-encapsulated actinomycin, at a dose usually toxic to mice, prolongs the mean survival time of mice bearing Ehrlich ascites tumor. (3) Liposomes containing actinomycin D are taken up by Ehrlich ascites cells, and the drug thus transported into the tumor cells is capable of inducing cell death.

Attempts made by various authors to reduce the toxicity of antitumor agents either by modifying the molecular structure, or by coupling with DNA or other molecules (10–13) have met with limited or no success. Liposome-encapsulation of antitumor agents is a novel approach which yields encouraging results in the case of actinomycin D and might become a promising method if exploited further with other tumor systems and with other antitumor agents.

*Summary.* Actinomycin D, when encapsulated within liposomes, is less toxic to mice than the nonencapsulated form. A single dose (0.75 mg/kg) or multiple doses (1 × 0.50, 4 × 0.25 mg/kg) significantly increased the mean survival time of mice inoculated with Ehrlich ascites tumor cells. Liposomes containing actinomycin D were found in tumor cells and cell degeneration and death were subsequently observed.

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