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Distribution of Etoposide-Loaded Hydrophilic Albumin Microspheres in Mice

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ABSTRACT

Hydrophilic albumin microspheres of etoposide were prepared by the emulsion polymerization technique using glutaraldehyde as the cross-linking agent. The microspheres prepared had a mean diameter of 1.5 μm. The microspheres were injected into mice by the intravenous route. In all, 12 mice were selected for the study, out of which 10 were given the drug-loaded microspheres and 2 were kept as solvent control. The mice were sacrificed after 24 hr and the accumulation of drug was determined in lungs, liver, and kidney.

INTRODUCTION

Macromolecular drug carrier systems have been developed in an attempt to alter tissue localization of drugs. The most utilized drug carrier is albumin as it meets many of the requirements of the ideal carrier given by Widder et al. (1). Albumin microspheres are biodegradable (2), nonimmunogenic (3), and capable of holding a wide variety of drug molecules due to its increased protein-binding capacity (4). But tissue localization and distribution of the drug will be largely dictated by the properties of the carrier (5). As many research groups have investigated albumin microspheres as the most utilized drug carriers in cancer chemotherapy, it was decided to select an anticancer drug as a drug candidate in our distribution study in mice.

Etoposide is a semisynthetic derivative of 4′-demethyl epipodophyltoxin, a naturally occurring compound found in the *Podophyllum peltatum* and the Indian species *Podophyllum Elaichi*. Chemically it is 4-demethyl epipodophyllotoxin-9-(4,6-*O*)-ethylidiene-D-glycupyranoside. It is indicated in the management of small-cell lung cancer, malignant lympoma, bladder cancer, and trophoblastic diseases. Its usual dose is 50 to 100 mg/m^2/day,

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and usually the treatment the treatment is continued for 5 days. After an IV injection, peak concentration of 30 μg/ml is achieved; this has a biphasic pattern of clearance with half-life of about 3 hr and 12 hr.

Hydrophilic albumin microspheres were selected to load the drug because they offer several advantages (4) over hydrophobic microspheres; they do not require the surfactants currently used to prepare the aqueous dispersions of hydrophobic microspheres (6), which may influence tissue interactions and drug release. The technique is different from other procedures in that a concentrated high molecular weight polymer is used to spheroidize the albumin. The object of the present study was to study the extent of drug accumulation in various organs of mice for hydrophilic microspheres of mean size less than 5 μm.

EXPERIMENTAL

Materials

As stated earlier, etoposide USP was the drug selected for loading the microspheres. Other materials were egg albumin flakes, glutaraldehyde, glycine, castor oil, linseed oil, toluene, and acetone.

Preparation of Hydrophilic Albumin Microspheres

The microspheres were prepared as follows (7,8): 2 ml of 20% egg albumin solution containing 7.5 mg of drug was prepared and added to a mixture of 15 ml castor oil and 10 ml toluene kept in a glass beaker, and stirred with a high-speed mixer. The speed was adjusted to obtain globules of the desired size, which was checked using an optical microscope.

Glutaraldehyde-saturated toluene solution was prepared by mixing equal volumes of glutaraldehyde and toluene. This was added dropwise to albumin dispersions with constant stirring for 4 hr. One milliliter of 1% glycine solution was added to cap any free aldehyde groups, and it was further stirred for 1 hr. Afterwards, the suspension of microspheres was washed free of oil with toluene. This was done in a high-speed centrifuge rotating at 2500 rpm. The centrifugation was carried out for 5 min, after which the supernate was removed and microspheres were resuspended in fresh toluene. This process was repeated 4 times. Afterwards the microspheres were suspended in acetone and centrifuged at 2500 rpm. The supernate acetone solution was removed and microspheres were resuspended in fresh acetone. This process was also repeated for 3 times. Finally the microspheres were suspended in 5 ml of deionized water and centrifuged for 5 min. The microspheres were poured into a paper dish for air drying at 25°C. Upon drying, yellow to yellowish-orange colored, free-flowing powder was obtained.

Size Distribution

Examination of the size distribution of hydrophilic albumin microspheres was carried out by scanning electron microscopy, using a Cambridge STEREOSCAN-100. Different magnifications were used. The diameters of 100 microspheres were measured and the average was calculated. The average diameter of the microspheres was found to be 1.287 μm, with a standard deviation of ±0.429 (Figs. 1 and 2).

In Vitro Drug Release

This study was carried out following the procedure described by Kim et al. (9). Etoposide-loaded microspheres, 50 mg, were taken in a 250-ml conical flask and 100 ml of isotonic normal saline was added. The flask was kept in shaker cum incubator maintained at 37°C samples, 5 ml , of the drug-releasing medium were withdrawn at various time intervals and replaced by the same volume of isotonic normal saline. Each sample was filtered through a membrane filter of pore size 0.2 to 0.45 μm under vacuum. The drug content was estimated by high-performance liquid chromatography (HPLC) (10) (Waters, model 484). The samples

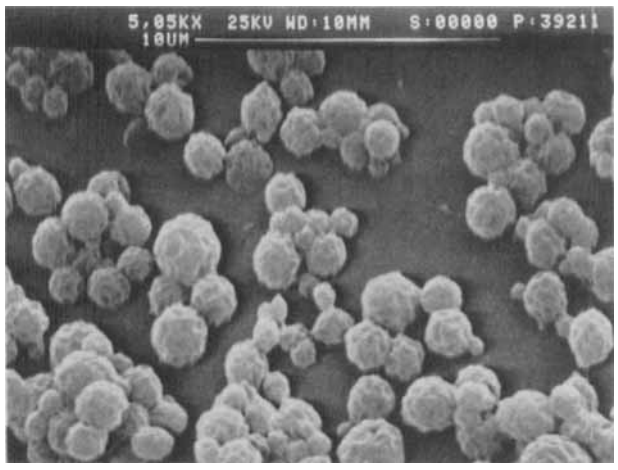

Figure 1. Scanning electron micrograph of etoposide-loaded albumin microspheres taken under magnification of 5740 times.

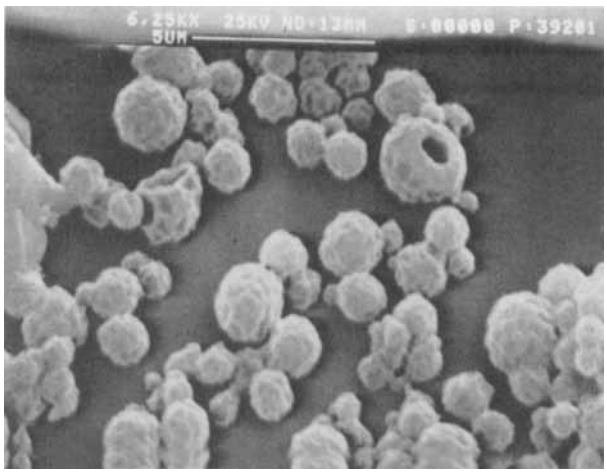

Figure 2. Scanning electron micrograph of etoposide-loaded albumin microspheres taken under magnification of 6250 times.

were withdrawn after 0.5, 1, 2, 4, 8, 16, and 24 hr. (see Table 1.)

Distribution Studies

Twelve healthy adult mice weighing 35 to 40 g were selected and fasted for 12 hr. Ten of the mice were given a dose of 2.5 mg of etoposide microspheres equivalent to 125 μm of etoposide. The remaining 2 mice were treated as solvent control and were injected intravenously with 1 ml/100 g of sterile normal saline solution (11).

After 24 hr, all 10 mice which were given the drug were sacrificed and their liver, lungs, and kidneys were isolated. The individual organs of each mouse was homogenized separately with 5 ml of methanol and centrifuged for 30 min. The supernatant layer was collected and analyzed for drug content by the HPLC method. (See Table 2.)

Table 1

In Vitro Release Study: Cumulative Percentage of Drug Release for Three Sample Batches

Time Interval, hr	Cumulative Percentage of Drug Release in Three Sample Batches		
	I	II	III
0.5	49.0	52.0	50.0
1.0	57.0	58.0	57.4
2.0	62.2	63.0	61.0
4.0	65.8	67.4	66.8
8.0	72.8	73.2	72.0
16.0	74.0	74.6	75.0
24.0	77.6	78.0	78.4

Table 2

Amount and Percentage of Drug Accumulated in Various Organs of Mice

Animal Number	Liver		Lung		Kidney	
	Amount of Drug Accumulated[a]	Percentage of Drug Accumulated	Amount of Drug Accumulated	Percentage of Drug Accumulated	Amount of Drug Accumulated	Percentage of Drug Accumulated
1	34.62	27.7%	38.2	30.6%	24.6	19.7%
2	28.68	22.9%	42.6	34.1%	28.0	22.4%
3	32.80	26.2%	40.0	32.0%	32.2	25.8%
4	30.93	24.7%	32.4	25.9%	30.8	24.6%
5	29.42	23.5%	40.5	32.4%	26.4	21.1%
6	26.40	21.1%	38.5	30.8%	21.2	25.0%
7	28.71	23.0%	36.2	29.0%	25.5	20.4%
8	25.60	20.5%	34.0	27.2%	27.0	21.6%
9	35.42	28.3%	43.4	34.7%	28.2	22.6%
10	33.72	27.0%	46.2	37.0%	23.1	18.5%
Average values	30.63	24.5%	39.2	31.4%	27.7	22.2%

Note. Each mouse received etoposide-loaded microspheres equivalent to 125 μg in 1 ml intravenously.
[a]Amount of drug accumulated in micrograms.

Estimation of Drug

The drug estimation was done by following the HPLC assay procedure (10). An HPLC model 484 (Waters Inc., USA) was used for this purpose, using a phenyl column.

The column had 3.9 mm internal diameter and was 300 mm in length. It was packed with 10-μm particles of Silicagel chemically loaded with phenyl groups. The mobile phase was dilute glacial acetic acid (1:100) containing 6.44 g sodium sulfate in 1000 ml.

The flow rate was adjusted so that a retention time of 20 min for etoposide was obtained.

RESULTS AND DISCUSSION

Electron scanning microscopy of hydrophilic albumin microspheres loaded with etoposide has shown that the microspheres formed were of spherical shape and smooth surface; their mean diameter was 1.287 ± 0.429. Since the standard deviation was very small, it can be said that process parameters established by our studies were able to control the particle size in a narrow range. The in vitro release of the drug from the microspheres has shown an interesting biphasic release. In the first half hour, 52% of the drug present in microspheres was released. Afterwards the drug release followed a steady pattern approximating zero-order release. The total percentage drug released after 24 hr was found to be 78%, which may be considered as satisfactory. The mechanism for the burst release in first 30 min can be attributed to the presence of some drug particles with imperfect albumin encapsulation.

The in vivo accumulation of the drug in different organs of mice has shown that the drug accumulates selectively in these organs to the extent of 78% after intravenous administration. It was found that 31.4% of the drug was accumulated in lungs, 24.5% in liver, and 22.2% in kidney. In other words, out of the total drug accumulated in these organs, 40% of the drug was accumulated in lungs.

These studies have established that albumin microspheres of etoposide offer distinct advantage over the IV injection of the pure drug itself because it has been reported that after an IV injection the concentration of the drug in blood and lungs is the same. But in the present studies a very high percentage of drug was accumulated in the lungs. Thus it can be concluded that the etoposide-loaded hydrophilic microspheres may be used with great advantage in treatment of lung cancer.

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