Effective Targeting of Solid Tumors in Patients With Locally Advanced Cancers by Radiolabeled Pegylated Liposomes

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ABSTRACT

The biodistribution and pharmacokinetics of 111 In-DTPA-labeled pegylated liposomes (IDLPL) were studied in 17 patients with locally advanced cancers. The patients received 65–107 MBq of IDLPL, and nuclear medicine whole body gamma camera imaging was used to study liposome biodistribution. The $t_{1/2}$ of IDLPL was 76.1 h. Positive tumor images were obtained in 15 of 17 studies (4 of 5 breast, 5 of 5 head and neck, 3 of 4 bronchus, 2 of 2 glioma, and 1 of 1 cervix cancer). The levels of tumor liposome uptake estimated from regions of interest on gamma camera images were approximately 0.5–3.5% of the injected dose at 72 h. The greatest levels of uptake were seen in the patients with head and neck cancers [33.0 ± 15.8% ID/kg (percentage of injected dose/kg)]. The uptake in the lung tumors was at an intermediate level (18.3 ± 5.7% ID/kg), and the breast cancers showed relatively low levels of uptake (5.3 ± 2.6% ID/kg). These liposome uptake values mirrored the estimated tumor volumes of the various tumor types (36.2 ± 18.0 cm$^3$ for squamous cell cancer of the head and neck, 114.5 ± 42.0 cm$^3$ for lung tumors, and 234.7 ± 101.4 cm$^3$ for breast tumors). In addition, significant localization of the liposomes was seen in the tissues of the reticuloendothelial system (liver, spleen, and bone marrow). One patient with extensive mucocutaneous AIDS-related Kaposi sarcoma was also studied according to a modified protocol, and prominent deposition of the radiolabeled liposomes was demonstrated in these lesions. An additional two patients with resectable head and neck cancer received 26 MBq of IDLPL 48 h before undergoing surgical excision of their tumors.

Samples of the tumor, adjacent normal mucosa, muscle, fat, skin, and salivary tissue were obtained at operation. The levels of tumor uptake were 8.8 and 15.9% ID/kg, respectively, with tumor uptake exceeding that in normal mucosa by a mean ratio of 2.3:1, in skin by 3.6:1, in salivary gland by 5.6:1, in muscle by 8.3:1, and in fat by 10.8:1. These data strongly support the development of pegylated liposomal agents for the treatment of solid tumors, particularly those of the head and neck.

INTRODUCTION

Liposomes were first described more than 30 years ago (1), and their subsequent development has resulted in the generation of a diverse array of formulations capable of entrapping a wide range of agents (reviewed in Ref. 2). The development of surface-modified liposomes in the last two decades has fuelled interest in the use of liposomes in cancer treatment (3–5). The discovery that incorporation of MPEG-derivatized lipids into liposome membranes results in prolonged circulation was a major advance in that it provided a safe synthetic compound that could be produced in large quantities (5). The presence of MPEG-derivatized (pegylated) lipids in the bilayer membrane of sterically stabilized liposomes effectively furnishes a steric barrier against interactions with plasma proteins and cell surface receptors that are responsible for the rapid intravascular destabilization/rupture and RES clearance seen after i.v. administration of conventional liposomes. As a result, pegylated liposomes have a prolonged circulation half-life, and the pharmacokinetics of any encapsulated agent are altered to conform to those of the liposomal carrier rather than those of the entrapped drug (6). Because the mechanism of tumor localization of pegylated liposomes is by means of extravasation through leaky blood vessels in the tumor (7, 8), prolonged circulation is likely to favor accumulation in the tumor by increasing the total number of passes made by the pegylated liposomes through the tumor vasculature.

Thus far, there have been no studies to address specifically the question of the ability of pegylated liposomes to target solid cancers. Preliminary imaging studies with conventional phospholipid vesicles in patients with cancer (9, 10) and AIDS-KS and non-Hodgkin lymphoma (11) have confirmed that conventional liposomes accumulate in human tumors. In recent years, the treatment of patients with AIDS-KS, breast cancers, and ovarian cancers with liposomal chemotherapy has given an

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indication of the potential value of this modality of therapy (12–16). In addition to influencing response rates, encapsulation within a pegylated lipidosome matrix modifies the toxicity of the agent in question. This phenomenon has been most thoroughly studied in the case of doxorubicin, in which case alopecia, vesicant activity, and cardiotoxicity are significantly reduced for the pegylated lipidosomal formulation (15, 17, 18). However, lipidosome-mediated alterations in the biodistribution and pharmacokinetics of entrapped agents may have deleterious, as well as beneficial, effects. Most notably, for pegylated lipidosomal doxorubicin, a novel dose-limiting form of skin toxicity known as palmar-plantar erythrodysaesthesia or hand-foot syndrome has been described (19). This side effect probably occurs as a result of extravasation of pegylated liposomes within the skin and the subsequent release of their contents.

New pegylated lipidosomal agents are under preclinical and Phase I/II clinical development in a range of tumor types (2, 20). In this study, we demonstrate targeting of IDLPL to a range of common solid tumors in patients with locally advanced cancers and extend the preliminary information on the normal tissue biodistribution and pharmacokinetics of these lipidosomes that has been reported previously (21). Such information will provide an important background to future clinical studies.

**PATIENTS AND METHODS**

**Biodistribution, Pharmacokinetic, and Imaging Study.** This study was approved by the Research Ethics Committee of Hammersmith Hospitals National Health Service Trust and the Administration of Radioactive Substances Advisory Committee. Seventeen patients (nine males and eight females) were studied. The median age of the patients was 59 (range 43–75) years. The malignant diagnoses were as follows: breast cancer (n = 5); SCCHN (n = 5); lung cancer (n = 4); high-grade glioma (n = 2); and cervix cancer (n = 1). All of the patients had biopsy-proven locally advanced tumors and good performance status (Karnofsky ≥ 70%). Written informed consent was obtained. Hematological and biochemical profiles were checked before the patients were enrolled into the study. These blood tests were repeated at the time of the final whole body scan to assess possible toxicity of this radiolabeled lipidosomal preparation. Patients were excluded from entry into the study if they met any of the following conditions: premenopausal, pregnant, or breastfeeding conditions; signs and symptoms of acute infection; invasive surgical procedure or radiotherapy to the tumor in the preceding 3 weeks; cytotoxic chemotherapy or cytokine treatment administered in the preceding 4 weeks; clinically significant abnormalities of hepatic or renal function; confusion, disorientation, and active major psychiatric illness; and previous radiotherapy to the site of the primary tumor or to clinically significant metastases. Eligible patients received 65–107 MBq (1.76–2.89 mCi) of radiolabeled pegylated liposomes diluted in 500 ml of 5% dextrose as an i.v. infusion over 30–45 min. Patients were observed throughout the infusion for adverse reactions, and vital signs were measured immediately after the infusion and daily thereafter for the first 4 days and again at 10 days.

In addition, a 45-year-old male patient with extensive mucocutaneous AIDS-KS (stage T1IxS1) participated in the study according to a modified protocol. He received 0.7 mCi (26 MBq) of IDLPL as an infusion in 250 ml of 5% dextrose.

**Surgical Study.** In two patients with surgically resectable SCCHN, the levels of uptake of IDLPL within tumor and adjacent normal tissues were determined to investigate the feasibility of using lipidosome-encapsulated radiosensitizers in the treatment of SCCHN. The study was approved by the Research Ethics Committee of Hammersmith Hospitals National Health Service Trust and the Administration of Radioactive Substances Advisory Committee. Patient 1 was a 54-year-old male with a T2N1M0 squamous cell cancer of the lateral border of the tongue. He underwent laser left hemiglossectomy and left subpramohyoid neck dissection. Patient 2 had a T3N2M0 squamous cell cancer of the left tongue base with nodal metastases and underwent a left neck dissection, mandibular swing and resection of tongue base. The patients received an infusion of 26 MBq (0.7 mCi) of IDLPL in 250 ml of 5% dextrose 48 h before surgery. Samples of the primary tumor, adjacent normal mucosa, salivary gland, sternocleidomastoid muscle, adipose tissue, and skin were obtained at the time of surgery.

**Pegylated Liposomes and Radiolabeling Protocol.** DTPA (Janssen Chimica, Geel, Belgium) was entrapped by SEQUUS Pharmaceuticals, Inc. (Menlo Park, CA) in a proprietary pegylated liposome matrix with the following lipid composition (values expressed in percentage molar ratio): hydrogenated soybean phosphatidylcholine (56.2%); cholesterol (38.3%); and N-(carbamoyl-MPEG 2000)-1,2-diestearoyl-sn-glycero-3-phosphoethanolamine sodium salt (5.3%). The liposomes were supplied, aliquoted in sterile 20-ml vials at −20°C, and were subsequently stored at this temperature until the time of use. The phospholipid doses received were 374 mg of hydrogenated soybean phosphatidylcholine and 128 mg of N-(carb-
amoyl-MPEG 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt/20-ml vial. Liposomes were labeled by the method described previously (22). Briefly, 2 ml of 111In-labeled oxine (Amersham International plc, Amersham, United Kingdom) were incubated with 20 ml of DTPA-containing pegylated liposomes for 60 min at room temperature. Subsequently, any residual unencapsulated 111In-labeled oxine was chelated by addition of EDTA (BDH Limited, Poole, United Kingdom) to promote its prompt excretion after i.v. injection. Entrapment of 111In within the pegylated liposomes was assayed by loading a 10-μl sample onto a 20-ml Sephadex G-50 column (Pharmacia, Uppsala, Sweden). Thirty consecutive 1-ml fractions were eluted with PBS, and the activity of each fraction was counted in a Canberra Packard Minaxi 5550 (Canberra Packard, Pangbourne, Berks, United Kingdom) gamma counter. Administration proceeded if the labeling efficiency was >90%.

Scintigraphy. All of the scans were performed on a MS2 dual-headed gamma camera (Siemens plc, Germany) using high resolution, medium energy collimators. All of the whole body images were acquired at 6 cm/min. Before injection of the radiolabeled pegylated liposomes, a whole body transmission scan was performed using a 57Co-labeled source (Amersham International, Amersham, United Kingdom) to allow correction for body surface area (calculated from height and weight). For the purpose of these estimations, it was assumed that 1 ml of blood and plasma weighed 1 g. Similarly, the total amount of radioactivity excreted/day in the urine was calculated from the volume of urine collected in that day.

Estimation of Liposome Uptake From ROI. The uptake of radiolabeled liposomes in tumor and in specific organs (liver, spleen, kidney, and lung) was estimated by measuring the total number of counts in identical ROI on the geometric mean (liver, spleen, kidney, and lung) images. Briefly, if A is the total count in the organ or tumor defined by the ROI, then the count from the anterior image (C_a) is given, to a good approximation, by:

$$C_a = Ae^{-\mu x}$$

where $\mu$ = the linear attenuation coefficient and x = organ depth. Similarly, the count from the posterior image (C_p) is given by:

$$C_p = Ae^{-\mu(L-x)}$$

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Blood</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>95.0 ± 11.8</td>
<td>100.9 ± 13.6</td>
</tr>
<tr>
<td>4</td>
<td>85.3 ± 9.5</td>
<td>90.8 ± 13.0</td>
</tr>
<tr>
<td>24</td>
<td>70.7 ± 9.2</td>
<td>76.5 ± 13.7</td>
</tr>
<tr>
<td>48</td>
<td>55.5 ± 9.3</td>
<td>59.3 ± 12.1</td>
</tr>
<tr>
<td>72</td>
<td>46.3 ± 9.5</td>
<td>49.0 ± 11.9</td>
</tr>
<tr>
<td>96</td>
<td>36.4 ± 9.2</td>
<td>39.1 ± 11.6</td>
</tr>
<tr>
<td>240</td>
<td>4.9 ± 5.1</td>
<td>4.9 ± 5.4</td>
</tr>
</tbody>
</table>

**Table 1** Blood and plasma clearance of 111In-DTPA-labeled pegylated liposomes in 17 patients with advanced cancers. Data are expressed as total percentage of injected activity (mean ± SD) present in blood and plasma at sampling time points.

Blood and plasma clearance of 111In-DTPA-labeled pegylated liposomes in 17 patients with advanced cancers. The measured radioactivity in the whole blood sample is identical to that in the plasma after centrifugation at 2000 rpm for 15 min.

SPECT and static imaging of ROI were performed as appropriate. The scans were viewed by an experienced assessor without prior knowledge of the diagnosis and without reference to previous radiological examinations. For the patient with AIDS-KS, whole body nuclear medicine scans were performed at 4, 24, 72, and 168 h. A preinjection transmission scan was not performed, and there was no attempt to estimate uptake in ROI. Blood, plasma, and urine pharmacokinetics were not performed. For the two patients in the surgical study, whole body and SPECT nuclear medicine scans were performed at 2 h (patient 1) and 20 h (patient 2) before surgery.

Blood, Plasma, and Urine Pharmacokinetics. Blood samples (10 ml) were taken into tubes containing anticoagulant (lithium heparin) at each of the above time points. Whole blood radioactivity was measured by counting triplicate 1-ml specimens of whole blood and standard dilutions (10^{-1} to 10^{-4}) of the injected liposomes in the gamma counter. The remainder of the blood sample was then centrifuged at 2000 rpm for 15 min to separate the cellular components from the plasma fraction. Triplicate 0.5-ml samples of plasma were taken, and their content of radioactivity was measured separately. In addition, serial 24-h urine collections were performed for 96 h, and the daily and cumulative urinary excretion of 111In in that time period was determined. The content of radioactivity in blood, plasma, and urine was initially expressed as a percentage of the injected dose/g of fluid. The total amount of radioactivity present at each time point in the blood and plasma was estimated by deriving the total blood volume and the plasma volume from standard nomograms using the patient’s sex and body surface area (calculated from height and weight). For the purpose of these estimations, it was assumed that 1 ml of blood and plasma weighed 1 g. Similarly, the total amount of radioactivity excreted/day in the urine was calculated from the volume of urine collected in that day.
where $L$ = patient thickness over the ROI. The geometric mean of the anterior and posterior counts is $\overline{C_a}$ = $\frac{C_a + C_p}{2}$. The square of the geometric mean of the counts is therefore:

$$C_a^2 \times C_p = A^2 \cdot e^{\mu L}. \quad (A)$$

The anterior and posterior counts from the transmission scan are given by:

$$S_a = S e^{-\mu L}$$

and

$$S_p = S$$

where $S$ = activity of known $^{57}$Co-labeled source. Thus, the attenuation term $e^{\mu L}$ in equation (A) equates to $S_p/S_a$. Substituting this in equation (A) gives:

$$A = \sqrt{(C_a \times C_p) / S_a} \quad (B)$$

To obtain an absolute measure of the uptake in MBq, a calibration factor (counts/MBq) was estimated using sheets of perspex as a tissue equivalent material. The count from a syringe containing the radiolabeled liposomes at a known activity was measured using the same principles as those used for the transmission and emission scans for the patients. Therefore, as shown in equation (B), knowledge of the geometry of the detailed distribution of radionuclide and of the linear attenuation coefficient were not required.

**Estimation of Tumor and Organ Uptake.** The data were initially calculated in the form of whole organ uptake values. In addition, an attempt was made to estimate the level of uptake as a % ID/kg of tissue. The mass of the individual organs for both men and women were obtained from standard texts: liver, 1.6 kg (male), 1.3 kg (female); spleen, 0.15 kg; kidney, 0.15 kg; and lung, 0.625 kg (right), 0.565 kg (left). In the case of the tumor, its diameter in three dimensions ($d_1$, $d_2$, and $d_3$) was estimated from the available clinical and radiological information, and the tumor volume was calculated by the following equation:

$$\text{tumor volume} = \pi l_6 d_1 \times d_2 \times d_3$$

For the purposes of this study, it was assumed that the relative density of the tumor tissue was 1.0 in all of the cases. Therefore, the tumor volume in ml was assumed to equate to the tumor mass in g.

**Measurement of Uptake of Radiolabeled Pegylated Liposomes in Tumor and Adjacent Normal Tissues in Patients with Head and Neck Cancer.** Samples of the resected tumor, adjacent mucosa (from two different sites within a hypothetical radiotherapy portal), salivary gland, skin, muscle, and fat were placed in weighed tubes and counted along with standard dilu-

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### Table 2  Urinary excretion of $^{111}$In.

Data are expressed both as total percentage of injected activity excreted in urine/day and cumulative total excreted during study period (mean ± SD).

<table>
<thead>
<tr>
<th>Day</th>
<th>Daily excretion</th>
<th>Cumulative excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>12.4 ± 4.9</td>
<td>12.4 ± 4.9</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.5 ± 0.6</td>
<td>13.9 ± 5.3</td>
</tr>
<tr>
<td>Day 3</td>
<td>2.3 ± 1.2</td>
<td>16.4 ± 6.1</td>
</tr>
<tr>
<td>Day 4</td>
<td>2.9 ± 0.8</td>
<td>18.3 ± 6.9</td>
</tr>
</tbody>
</table>

### Table 3  Patient details: histology, stage, and results of gamma camera imaging and estimated tumor uptake from ROI analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor</th>
<th>Stage</th>
<th>Whole body scan</th>
<th>SPECT</th>
<th>Total % injected dose$^a$</th>
<th>% ID/kg$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SCC bronchus</td>
<td>T4N0M0</td>
<td>Positive</td>
<td>Positive</td>
<td>1.7</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>SCC bronchus</td>
<td>T4N0M0</td>
<td>Positive</td>
<td>Positive</td>
<td>1.6</td>
<td>25.4</td>
</tr>
<tr>
<td>3</td>
<td>Breast (ductal)</td>
<td>T4N2M1</td>
<td>Negative</td>
<td>Negative</td>
<td>3.5</td>
<td>46.8</td>
</tr>
<tr>
<td>4</td>
<td>SCCHN</td>
<td>T3N2M0</td>
<td>Positive</td>
<td>Positive</td>
<td>0.3</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>Breast (ductal)</td>
<td>T4N1M0</td>
<td>Positive</td>
<td>Positive</td>
<td>1.5</td>
<td>3.9</td>
</tr>
<tr>
<td>6</td>
<td>Breast (ductal)</td>
<td>T4N2M1</td>
<td>Positive</td>
<td>Positive</td>
<td>1.7</td>
<td>9.5</td>
</tr>
<tr>
<td>7</td>
<td>Breast (ductal)</td>
<td>T3N2M0</td>
<td>Positive</td>
<td>Positive</td>
<td>0.7</td>
<td>24.2</td>
</tr>
<tr>
<td>8</td>
<td>SCCHN</td>
<td>T4N0M0</td>
<td>Positive</td>
<td>Positive</td>
<td>1.0</td>
<td>32.0</td>
</tr>
<tr>
<td>9</td>
<td>SCCHN</td>
<td>T3N1M0</td>
<td>Positive</td>
<td>Positive</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>SCC cervix</td>
<td>FIGO IIIB</td>
<td>Negative</td>
<td>Positive</td>
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<td>NA</td>
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<tr>
<td>11</td>
<td>Breast (ductal)</td>
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<td>Positive</td>
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<td>5.2</td>
</tr>
<tr>
<td>12</td>
<td>SCC bronchus</td>
<td>T2N0M1</td>
<td>Negative</td>
<td>Negative</td>
<td>0.6</td>
<td>9.0</td>
</tr>
<tr>
<td>13</td>
<td>SCCHN</td>
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<td>Positive</td>
<td>1.6</td>
<td>53.0</td>
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<tr>
<td>14</td>
<td>SCCHN</td>
<td>T3N0M0</td>
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<td>Positive</td>
<td>2.6</td>
<td>16.7</td>
</tr>
<tr>
<td>15</td>
<td>SCC bronchus</td>
<td>T3N0M1</td>
<td>Positive</td>
<td>Positive</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>Glioma (AA)</td>
<td>Inoperable</td>
<td>Negative</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>17</td>
<td>Glioma (GBM)</td>
<td>Inoperable</td>
<td>Negative</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$ Tumor uptake determined from ROI on 72 h whole body scan.

$^b$ Percentage injected dose/kg calculated from estimated tumor volume.

SCC, squamous cell cancer; AA, anaplastic astrocytoma (grade III); GBM, glioblastoma multiforme (grade IV); NA, not assessable (tumor uptake was only measurable from whole body scans).
tions ($10^{-1}$ to $10^{-4}$) of the injected liposomes in the gamma counter. The tubes were then reweighed, and their content of radiolabeled liposomes was expressed in terms of % ID/kg.

In Vivo Stability of IDLPL in the Circulation. The stability of IDLPL in the circulation was assessed by taking blood samples at 24, 48, 72, and 96 h into tubes containing anticoagulant and centrifuging them at 2000 rpm for 15 min. Samples of 500 μl of plasma were taken, filtered through a 0.2-μm filter (Acrodisc; Gelman Science, Inc., Ann Arbor, MI), and run on a Superose-6 FPLC column. Eighty fractions of 0.5 ml were collected and counted in the gamma counter. As a standard, a 100-μl sample of the radiolabeled liposomes was run on the same column.

RESULTS

Labeling Efficiency. The median liposome labeling efficiency as assessed by running samples on a Sephadex G-50 column was 95.0% (range 89.8–97.0%). A representative example of such a separation is shown in Fig. 1. The liposome-encapsulated $^{111}$In-DTPA was collected in fractions 6 to 12, and the peak of unencapsulated EDTA-bound $^{111}$In was collected in fractions 16 to 21. For the purpose of calculating the labeling efficiency, the counts collected in fractions 6 to 12 were expressed as a percentage of the total number of counts in all of the 30 fractions.

Liposome Biodistribution and Pharmacokinetics. The estimated total levels of IDLPL measured in whole blood and
plasma are presented in Table 1. Fig. 2 shows the clearance of the IDLPL from the blood and plasma over the 240-h period of the study. It can be seen that all of the $^{111}$In was contained in the plasma fraction, which suggested that none of the circulating radioactivity was associated with blood cells.

The excretion half-life ($t_{1/2}$) of the IDLPL was determined by fitting the whole blood data between 4 h and 96 h to the mono-exponential decay equation:

$$y = Ae^{-kt}$$

where $t_{1/2} = 0.693/k$. Using this method, the $t_{1/2B}$ was found to be 76.1 h. The goodness of fit was within 95% of expected limits for a correct model of the data. The data available did not permit accurate derivation of a $t_{1/2A}$.

The median cumulative urinary excretion of $^{111}$In over the first 96 h was 19.5% (range 3.5–28.4%). The time course of urinary excretion of $^{111}$In is shown in Table 2. As can be seen, the majority of the $^{111}$In was excreted in the first 24 h. A significant proportion of this was undoubtedly because of rapid

Fig. 4 Gamma camera images at 72 h of patient with T3N0M0 squamous cell cancer of the tongue base (patient 14). A, whole body gamma camera scan revealing tongue base tumor (Tu), cardiac blood pool (CP), liver (L), and spleen (Spl). In addition, radioactivity localized to the bowel is seen. B, sagittal SPECT image. C, coronal SPECT image. D, transverse SPECT image.
excretion of the unencapsulated EDTA-bound radioactivity. The remainder of this early urinary excretion was probably because of initial intravascular rupture or RES degradation of damaged or defective liposomes. A small percentage of the injected radioactivity was excreted on each of the subsequent days, suggesting slow degradation of the pegylated liposomes within the blood or tissues and gradual elimination of their content of $^{111}$In-DTPA.

Results of Scintigraphy. Analysis of the sequential images obtained in each patient revealed a consistent pattern of biodistribution. A strong early blood pool image was seen at 30 min, at which time activity, presumably because of EDTA-bound $^{111}$In, was also clearly visualized in the kidneys and urinary bladder. The blood pool signal declined slowly, in keeping with the prolonged circulation half-life. Normal organ uptake was seen most prominently in the RES of the spleen and liver, although low-level uptake was also seen in the nasal mucosa, in the bowel (probably because of hepatic excretion of the radioisotope), and diffusely in the bone marrow.

Positive tumor images were obtained in a total of 18 of 20 studies. For the 17 patients who participated in the biodistribution and pharmacokinetic study according to the full protocol, the tumor was seen in 15 patients (Table 3). Clear visualization of the tumors was not usually obtained until 48–72 h after injection because of the high blood background signal. In 12 of the 17 patients, the tumor was clearly seen on the whole body images and, in an additional 3 patients (2 gliomas, 1 cervical cancer), SPECT scans of the ROI were required to identify the tumor. Fig. 3, Fig. 4, and Fig. 5 show representative scans of three patients, one each with breast, lung, and head and neck cancer. Fig. 6 shows the whole body scans at 4, 24, 72, and 168 h in the patient with AIDS-KS in whom a large number of lesions over the left foot, left calf, thigh, arms, and face were clearly delineated.
In both patients who received radiolabeled liposomes before surgery, the tumor was visualized on the whole body and SPECT scans. The amount of injected activity, however, precluded an accurate estimate of the level of liposome uptake in the tumor from the gamma camera images.

Estimation of Tumor and Organ Uptake from Regions of Interest. In the 12 tumors that were clearly seen on the whole body scans, the maximum level of uptake varied from 0.3 to 3.6% of the injected dose at 72 h. The mean estimated tumor volumes for the various tumor groups were as follows: 36.2 ± 18.0 cm$^3$ for SCCHN; 114.5 ± 42.0 cm$^3$ for lung tumors; and 234.7 ± 101.4 cm$^3$ for breast tumors. When the liposome uptake data were expressed in terms of the % ID/kg of tumor, the results varied between 2.7 and 53.0% ID/kg. When these data were analyzed separately according to the site of the primary tumor site, there was considerable variation (Fig. 7). The greatest levels of uptake were seen in the SCCHN (33.0 ± 15.8% ID/kg). The uptake in the lung tumors was at an intermediate level (18.3 ± 5.7% ID/kg), and the breast cancers showed relatively low levels of uptake (5.3 ± 2.6% ID/kg). These data were calculated only for the tumors that were visualized on the whole body scans. The two patients with negative scans, one each with breast and lung cancer, were not included in this analysis.

Normal organ uptake in the liver, spleen, kidneys, and lungs was estimated for all of the 17 patients. The data for whole organ uptake are presented in Table 4. As can be seen, absolute uptake was greatest in the liver, which reached a peak at 4 h and remained virtually constant throughout the period of the study. The uptake in the spleen increased throughout the first 3 days to reach a peak value at 72 h. These data demonstrate that, although pegylated liposomes are engineered to evade the RES, there is still a significant amount of clearance via this route. When the uptake is expressed in terms of % ID/kg of tissue, the relative uptake in the spleen exceeds that in the liver by a factor of 4 (Table 4). The activity localized in the kidneys was greatest at the 0.5-h scan and showed a gradual decline during the study period. In addition to tissue localization of radiolabeled lipo-
Table 4  Normal organ uptake of $^{111}$In-DTPA pegylated liposomes as determined by regions of interest on the whole body gamma camera scans. The data are given both for whole organ uptake and for estimated uptake/kg. The weight of each organ was derived from standard texts, and the following values were used: liver, 1.6 kg (male); 1.3 kg (female); spleen, 0.15 kg; kidney, 0.15 kg; lung, 0.595 kg [the mean of 0.625 kg (right) and 0.565 kg (left)].

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>% whole organ</th>
<th>% ID/kg</th>
<th>% whole organ</th>
<th>% ID/kg</th>
<th>% whole organ</th>
<th>% ID/kg</th>
<th>% whole organ</th>
<th>% ID/kg</th>
<th>% whole organ</th>
<th>% ID/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>11.2 ± 2.7</td>
<td>7.6 ± 1.8</td>
<td>3.6 ± 1.7</td>
<td>24.0 ± 11.3</td>
<td>5.6 ± 1.4</td>
<td>9.4 ± 2.6</td>
<td>1.6 ± 0.8</td>
<td>10.7 ± 5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11.7 ± 1.9</td>
<td>8.0 ± 1.3</td>
<td>3.8 ± 1.7</td>
<td>25.3 ± 11.3</td>
<td>5.2 ± 1.3</td>
<td>8.7 ± 2.2</td>
<td>1.4 ± 0.7</td>
<td>9.3 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>11.5 ± 2.4</td>
<td>7.8 ± 1.6</td>
<td>4.6 ± 1.8</td>
<td>30.7 ± 12.0</td>
<td>4.7 ± 1.2</td>
<td>7.9 ± 2.0</td>
<td>1.4 ± 0.7</td>
<td>9.3 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>11.2 ± 2.4</td>
<td>7.6 ± 1.6</td>
<td>4.9 ± 2.0</td>
<td>32.7 ± 13.3</td>
<td>4.2 ± 1.2</td>
<td>7.1 ± 2.0</td>
<td>1.3 ± 0.7</td>
<td>8.7 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>10.9 ± 2.5</td>
<td>7.4 ± 1.7</td>
<td>5.1 ± 2.2</td>
<td>34.0 ± 14.7</td>
<td>3.8 ± 1.1</td>
<td>6.4 ± 1.8</td>
<td>1.3 ± 0.7</td>
<td>8.7 ± 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>10.3 ± 2.8</td>
<td>7.0 ± 1.9</td>
<td>4.9 ± 2.3</td>
<td>32.8 ± 14.2</td>
<td>3.4 ± 1.1</td>
<td>5.7 ± 1.8</td>
<td>1.2 ± 0.7</td>
<td>8.0 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>7.6 ± 1.7</td>
<td>5.2 ± 1.2</td>
<td>3.4 ± 1.8</td>
<td>22.7 ± 12.1</td>
<td>2.0 ± 0.7</td>
<td>3.4 ± 1.2</td>
<td>0.65 ± 0.38</td>
<td>4.3 ± 2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5  Tumor and adjacent normal tissue localization of $^{111}$In-DTPA-labeled pegylated liposomes in two patients with operable SCCHN.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% ID/kg</th>
<th>T:N Ratio</th>
<th>% ID/kg</th>
<th>T:N Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>8.8</td>
<td>1.0</td>
<td>15.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Tongue mucosa</td>
<td>4.5</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>6.2</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palatal mucosa</td>
<td></td>
<td></td>
<td>5.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>1.8</td>
<td>4.9</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Fat</td>
<td>1.9</td>
<td>4.7</td>
<td>0.9</td>
<td>16.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.5</td>
<td>5.8</td>
<td>1.5</td>
<td>10.8</td>
</tr>
<tr>
<td>Skin</td>
<td>4.2</td>
<td>2.1</td>
<td>3.1</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Toxicity Evaluation. One of the patients suffered an acute reaction during the liposome infusion. This consisted of flushing of the face, chest, and upper extremities associated with some chest tightness and back pain. This reaction was associated with mild tachycardia but no alteration in blood pressure. The reaction abated within a few min after discontinuation of the infusion and did not recur when the infusion was restarted at a slower rate. This reaction has been reported previously in the clinical studies (13, 14) of pegylated liposome-encapsulated doxorubicin. Otherwise, there were no other adverse reactions attributable to the liposome infusion, and the repeat hematological and biochemical profiles performed at 10 days showed no significant changes.

DISCUSSION

The development of targeted therapies for cancer has remained an elusive goal. The optimism that followed the description of monoclonal antibodies (23) has not been translated into effective targeted treatments despite concerted efforts using radioisotopes, drugs, and toxins (24). The use of pegylated liposomes as targeting vehicles for anticancer agents represents an exciting new avenue of research, with the twin attractions of delivering increased drug concentrations preferentially to tumor sites while having the potential to spare dose-limiting normal tissues from the toxic effects of the free drug. The results of this study suggest that pegylated liposomes are effective as a means of targeting a variety of solid tumors. Although unencapsulated $^{111}$In-DTPA was not injected as a control, previous studies in tumor-bearing mice have shown that unencapsulated $^{111}$In-DTPA is cleared very rapidly from the blood after i.v. injection (22) and from tumor tissue after intratumoral injection (25). Furthermore, previous studies (22) have shown that incubation of unencapsulated $^{111}$In-DTPA with plasma does not result in transchelation of the isotope onto serum proteins. These data strongly suggest that the radioactivity detected in the tumor was...
delivered by liposomes and not by some other radiolabeled moiety.

Although this study has clearly demonstrated that pegylated liposomes are able to accumulate in solid tumors at high concentrations and remain there for prolonged periods, there was considerable heterogeneity of uptake of the liposomes both between different tumor types and between different patients with the same tumor type. The differences are accentuated if the liposome uptake is expressed as a function of a relative unit of tumor mass. The levels of uptake seen in the breast tumors \( (5.3 \pm 2.6\% \text{ ID/kg}) \) were considerably lower than those seen in the lung \( (18.3 \pm 5.7\% \text{ ID/kg}) \) and head and neck tumors \( (33.0 \pm 15.8\% \text{ ID/kg}) \). It is unclear why certain tumors showed higher levels of uptake than others and, indeed, why two of the tumors (1 breast cancer, 1 lung cancer) were not seen on either whole body or SPECT imaging. Detailed information regarding the histology and vascular architecture of the tumors was not available, although it is intriguing to hypothesize that differences in the density and structural and functional integrity of the tumor neovasculature may be responsible for at least some of the variability. Koukourakis et al. (26) have studied this issue in patients with lung and head and neck cancers who received \(^{99m}\text{Tc-DTPA}\)-labeled pegylated liposomes and demonstrated that microvessel density assessed with anti-CD31 monoclonal antibody staining directly correlated with the degree of the liposome accumulation. In addition, the size of the tumors and the presence of areas of poor vascularization, or even necrosis, may have influenced the results. There is evidence from xenograft studies that larger tumors have lower levels of liposome uptake and are more likely to contain necrotic areas (27). In terms of estimated tumor mass, the breast tumors were the largest, and the SCCHN were the smallest. These data mirrored the results for tumor liposome uptake shown in Fig. 7. However, this relationship was not absolute, and tumors of different histological types but similar sizes had different levels of liposome uptake. Another potentially important factor is the presence or

### Table 6

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
</table>
| Percentage
    encapsulated
    \(^{111}\text{In-DTPA}\) | 95.4  | 93.4  | 94.9  | 95.2  |
| Percentage
    unencapsulated
    \(^{111}\text{In-DTPA}\) | 4.6   | 6.6   | 5.1   | 4.8   |

Fig. 8  Superose-6 FPLC separation of patient plasma 24, 48, 72, and 96 h after injection of \(^{111}\text{In-DTPA}\)-labeled pegylated liposomes.
absence of associated inflammation. Pegylated liposomes have been shown to localize efficiently to inflamed areas in a number of animal studies (28, 29). It is interesting to note that a common feature of patients with locally advanced SCCHN and lung cancers is the presence of tissue inflammation with or without superadded infection. This may well have accounted, in part, for the higher levels of tumor localization in these primary sites.

This heterogeneity of liposome uptake in the different tumor types may explain the results of a Phase II study of pegylated liposome-encapsulated doxorubicin in patients with breast cancer in which the response rate was 31%. This is approximately the same as would be expected for free doxorubicin in this disease (15). However, these data are in keeping with the findings of this study, which suggest that some breast cancers will fail to be targeted by pegylated liposomes and that others will take up subtherapeutic doses of drug. In contrast, the prominent liposome uptake in head and neck and lung cancers suggests that these tumors might be suitable targets for liposomally targeted therapies. The incorporation of a pretreatment IDLPL uptake scan into future Phase II studies offers the possibility of testing whether this investigation can predict the likelihood of a response to treatment in different groups of patients.

This study also underlines the interpatient variability in pharmacokinetics of pegylated liposomes, with the $t_{1/2}$ varying from 40 to 100 h. Such differences may have important clinical implications in terms of both the efficacy and toxicity of therapy. From first principles, prolonged circulation would be expected to increase liposome accumulation in tumors and, hence, improve response rates. However, toxicity may also be a function of liposomal longevity in the circulation, because this would also be expected to increase exposure of dose-limiting tissues (bone marrow, skin, and mucous membranes) to the encapsulated agent.

In addition to the delivery of cytotoxic drugs, pegylated liposomes have the potential to function as a carrier vehicle for a range of anticancer agents to solid tumors, facilitating exploration of a range of novel targeted strategies. One potential application that is currently under investigation in our laboratory is liposomal entrapment of drugs that sensitize cells to the effects of ionizing radiation. A variety of radiosensitizers have impressive in vitro activities but cause significant local and systemic toxicity, which has hindered their clinical utility. In addition, the lack of tumor targeting means that there may be equal radiosensitization in both the tumor and adjacent normal tissues. Entrapment of radiosensitizers within pegylated liposomes offers the prospect of dramatically altering the biodistribution and pharmacokinetics of these agents, thus increasing the tumor concentration of the radiosensitizing drug as compared with the adjacent normal tissues. This phenomenon is demonstrated clearly in the clinical images and suggests that an advantageous differential radiosensitization effect could be achieved in the tumor without unacceptable local normal tissue toxicity. Furthermore, the limited data available from the two patients who underwent surgical excision of their tumors revealed that the uptake of the liposomes into the tumor does exceed that in the adjacent dose-limiting normal structures. Indeed, the tumor:normal tissue ratios for skeletal muscle were very similar to those obtained in a previous study in two patients with bone metastases from breast cancer (30). Most importantly, the mean ratio of uptake in the tumor compared with the mucosa was 2.4:1 (range 1.4 to 3.2), suggesting that targeted delivery of liposome-entrapped radiosensitizers may yield a preferential radiosensitizing effect in the tumor. Alteration of the biodistribution and pharmacokinetics of the drug also has the potential to reduce systemic drug toxicity, perhaps allowing greater doses of the radiosensitizer to be delivered. Preclinical studies of such liposomally entrapped radiosensitizing agents are in progress in our laboratory. In addition, the high levels of IDLPL delivered to solid tumors, especially those of the head and neck, raise the possibility that pegylated liposomes containing β-emitting radiopharmaceuticals might be capable of delivering a therapeutic radiation boost to tumors in an analogous manner to that under investigation for radiolabeled monoclonal antibodies (31). Detailed studies are in progress to examine the feasibility of this approach.

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REFERENCES


Pegylated Liposome Targeting of Solid Cancers


Clinical Cancer Research

Effective Targeting of Solid Tumors in Patients With Locally Advanced Cancers by Radiolabeled Pegylated Liposomes

Kevin J. Harrington, Sima Mohammadtaghi, Paul S. Uster, et al.


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