Pegylated Liposomes Have Potential as Vehicles for Intratumoral and Subcutaneous Drug Delivery

Kevin J. Harrington, Gail Rowlinson-Busza, Konstantinos N. Syrigos, Paul S. Uster, Richard G. Vile, and J. Simon W. Stewart

Molecular Cancer Research

ABSTRACT

The potential value of intratumoral or s.c. injections of pegylated liposomes as locoregionally targeted therapy of tumors and their draining lymph nodes was assessed in nude mice as part of an ongoing program aimed at developing pegylated liposomal radiosensitizers for the treatment of head and neck cancers. Animals received $^{111}$In-labeled diethylenetriaminepentaacetic acid (DTPA), either encapsulated in pegylated liposomes (IDLPL) or in the unencapsulated form ($^{111}$In-DTPA), as intratumoral or s.c. injections, and the local retention, locoregional nodal drainage, and systemic biodistribution were measured. After intratumoral injections, IDLPL were effectively retained in the tumor with an area under the curve (AUC) between 1 and 96 h of 2,574.4% injected dose per gram hour (%ID/g-h). The corresponding value for $^{111}$In-DTPA was 204.4%ID/g-h. Accumulation of IDLPL was seen in ipsilateral lymph nodes. The maximal ipsilateral:contralateral node ratios were 8:1 (2.2 versus 0.27%ID/g) for inguinal nodes at 24 h and 19:1 (2.5 versus 0.13%ID/g) for axillary nodes at 48 h. Unencapsulated $^{111}$In-DTPA showed no evidence of accumulation in locoregional nodes. After s.c. injection, IDLPL were cleared slowly from the injection site with an AUC between 1 and 192 h of 24,051.1%ID/g-h. Unencapsulated $^{111}$In-DTPA was cleared rapidly with an AUC between 1 and 192 h of 46.4%ID/g-h. Again, significant levels of IDLPL were detected in the ipsilateral locoregional nodes, with ipsilateral:contralateral ratios of 121:1 (57.9 versus 0.48%ID/g) at 24 h (inguinal nodes) and 17:1 (5.2 versus 0.3%ID/g) at 72 h (axillary nodes). There was no retention of unencapsulated $^{111}$In-DTPA in the draining nodes. Locoregional administration of pegylated liposomal radiosensitizers may be a useful approach for targeted therapy of head and neck tumors and their nodal metastases.

INTRODUCTION

Pegylated liposomes were initially developed with the primary goal of evading rapid clearance by the reticuloendothelial system, thus allowing them to remain in the circulation for prolonged periods after i.v. injection (1). This property of pegylated liposomes has been shown to result in effective tumor targeting (2–4) and therapeutic efficacy (5) in a number of animal models. Furthermore, in clinical studies the favorable pharmacokinetics and biodistribution of pegylated liposomal doxorubicin have been shown to translate to significant activity against AIDS-related Kaposi’s sarcoma (6, 7) and against ovarian (8) and breast cancers (9).

Although the main thrust of development of pegylated liposome-encapsulated therapeutic agents has focused on systemic administration, the ability to encapsulate a range of agents stably in pegylated liposomes and the relative lack of direct toxicity after accidental local administration (10) suggests that they may also have potential applications in the sphere of locoregional drug-targeting strategies. SCCHN, which is characterized by a natural history of local progression and locoregional nodal spread, may serve as an ideal target for such an approach. Thus far, only limited attention has been paid to the potential worth of locoregional depot delivery of liposomal therapeutic agents. Administration of various pegylated and nonpegylated liposomal agents via the i.p. (11–18), intrapleural (19), and intrathecal (20) routes has been shown to enhance local efficacy and to reduce systemic toxicity in a number of preclinical and clinical studies. By analogy, in the sphere of locoregional therapy targeted against a primary tumor and lymph node metastases, direct intratumoral and s.c. administration may be worth additional evaluation. In this article, each of these routes has been examined in detail with the aim of defining potential therapeutic roles. In particular, these data have been discussed in the context of delivery of liposome-encapsulated radiosensitizing agents.

MATERIALS AND METHODS

Animal Model

Female nude mice of mixed genetic backgrounds were used in all of the experiments. The animals were bred under specific pathogen-free conditions at the Imperial Cancer Research Fund Animal Breeding Unit (South Mimms, Herts.
used for the experiment 17–21 days after tumor inoculation. For injected s.c. into the right flank of the mice. The animals were and 5
trypsin/EDTA 0.02%, a single-cell suspension was prepared, and 5 × 10^6 tumor cells in 0.1 ml of culture medium were injected s.c. into the right flank of the mice. The animals were used for the experiment 17–21 days after tumor inoculation. For

Table 1  Biodistribution of 111In-DTPA pegylated liposomes in nude mice after intratumoral injection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>4.5 ± 4.2</td>
<td>9.4 ± 8.9</td>
<td>2.1 ± 0.8</td>
<td>3.4 ± 2.1</td>
<td>0.90 ± 0.55</td>
<td>0.64 ± 0.42</td>
</tr>
<tr>
<td>Blood</td>
<td>0.066 ± 0.029</td>
<td>0.59 ± 0.95</td>
<td>0.27 ± 0.30</td>
<td>0.036 ± 0.029</td>
<td>0.014 ± 0.005</td>
<td>0.018 ± 0.008</td>
</tr>
<tr>
<td>Tumor</td>
<td>76.4 ± 50.7</td>
<td>70.0 ± 43.1</td>
<td>34.7 ± 8.8</td>
<td>22.5 ± 10.1</td>
<td>12.8 ± 13.9</td>
<td>15.0 ± 12.3</td>
</tr>
<tr>
<td>IILN</td>
<td>1.2 ± 0.9</td>
<td>3.1 ± 2.7</td>
<td>2.2 ± 2.8</td>
<td>0.80 ± 0.72</td>
<td>3.2 ± 3.0</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>CILN</td>
<td>0.53 ± 0.54</td>
<td>1.0 ± 0.2</td>
<td>0.27 ± 0.08</td>
<td>0.28 ± 0.35</td>
<td>0.49 ± 0.31</td>
<td>0.63 ± 0.54</td>
</tr>
<tr>
<td>IALN</td>
<td>3.2 ± 4.5</td>
<td>7.2 ± 5.6</td>
<td>3.0 ± 4.0</td>
<td>2.5 ± 3.7</td>
<td>2.5 ± 1.8</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>CALN</td>
<td>0.31 ± 0.10</td>
<td>0.81 ± 0.35</td>
<td>0.24 ± 0.02</td>
<td>0.13 ± 0.11</td>
<td>0.27 ± 0.26</td>
<td>0.51 ± 0.45</td>
</tr>
<tr>
<td>Liver</td>
<td>0.9 ± 1.5</td>
<td>1.6 ± 0.9</td>
<td>0.75 ± 0.47</td>
<td>0.51 ± 0.55</td>
<td>1.2 ± 0.9</td>
<td>1.9 ± 1.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.12 ± 0.13</td>
<td>1.5 ± 1.0</td>
<td>0.51 ± 0.34</td>
<td>0.54 ± 0.64</td>
<td>1.3 ± 0.8</td>
<td>1.9 ± 1.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.26 ± 0.11</td>
<td>0.33 ± 0.21</td>
<td>0.34 ± 0.13</td>
<td>0.44 ± 0.21</td>
<td>0.67 ± 0.21</td>
<td>0.74 ± 0.32</td>
</tr>
<tr>
<td>Lung</td>
<td>0.074 ± 0.034</td>
<td>0.36 ± 0.27</td>
<td>0.11 ± 0.09</td>
<td>0.032 ± 0.034</td>
<td>0.058 ± 0.022</td>
<td>0.078 ± 0.033</td>
</tr>
</tbody>
</table>

* IILN, ipsilateral inguinal lymph node; IALN, ipsilateral axillary lymph node; CILN, contralateral inguinal lymph node; CALN, contralateral axillary lymph node.

Table 2  Biodistribution of 111In-DTPA in nude mice after intratumoral injection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>45.7 ± 27.5</td>
<td>92.4 ± 62.4</td>
<td>0.87 ± 0.85</td>
<td>0.24 ± 0.21</td>
<td>0.19 ± 0.22</td>
<td>0.057 ± 0.036</td>
</tr>
<tr>
<td>Blood</td>
<td>0.22 ± 0.05</td>
<td>0.07 ± 0.05</td>
<td>0.003 ± 0.002</td>
<td>0.000± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>Tumor</td>
<td>66.3 ± 27.2</td>
<td>12.0 ± 9.0</td>
<td>1.3 ± 0.7</td>
<td>0.95 ± 0.31</td>
<td>0.27 ± 0.29</td>
<td>0.36 ± 0.33</td>
</tr>
<tr>
<td>IILN</td>
<td>0.32 ± 0.16</td>
<td>0.11 ± 0.11</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>CILN</td>
<td>0.65 ± 0.56</td>
<td>0.13 ± 0.11</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>IALN</td>
<td>0.37 ± 0.11</td>
<td>0.10 ± 0.22</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>CALN</td>
<td>0.40 ± 0.20</td>
<td>0.25 ± 0.22</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
<tr>
<td>Liver</td>
<td>0.094 ± 0.033</td>
<td>0.054 ± 0.028</td>
<td>0.040 ± 0.009</td>
<td>0.030 ± 0.008</td>
<td>0.026 ± 0.009</td>
<td>0.021 ± 0.013</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.070 ± 0.017</td>
<td>0.028 ± 0.025</td>
<td>0.016 ± 0.011</td>
<td>0.024 ± 0.025</td>
<td>0.025 ± 0.029</td>
<td>0.023 ± 0.029</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.80 ± 0.20</td>
<td>0.47 ± 0.24</td>
<td>0.45 ± 0.11</td>
<td>0.40 ± 0.15</td>
<td>0.34 ± 0.16</td>
<td>0.17 ± 0.16</td>
</tr>
<tr>
<td>Lung</td>
<td>0.18 ± 0.05</td>
<td>0.06 ± 0.04</td>
<td>0.005 ± 0.004</td>
<td>0.023 ± 0.002</td>
<td>0.009 ± 0.011</td>
<td>0.005 ± 0.002</td>
</tr>
</tbody>
</table>

* IILN, ipsilateral inguinal lymph node; IALN, ipsilateral axillary lymph node; CILN, contralateral inguinal lymph node; CALN, contralateral axillary lymph node.

United Kingdom). Thereafter, the animals were transferred to the Biological Services Unit at the Imperial College of Science, Technology, and Medicine, Hammersmith Hospital; housed in sterile filter-top cages on sterile bedding; and maintained on an irradiated diet and autoclaved, acidified water (pH 2.8) ad libitum.

For the studies of intratumoral injection, mice that bore human KB head and neck cancer xenograft tumors (21) were used. The xenograft tumors were established as follows. KB tumor cells were grown to confluence in vitro in 175-cm² tissue culture flasks (Falcon, Lincoln Park, NJ) in RPMI 1640 containing penicillin 100 units/ml and streptomycin 100 µg/ml, supplemented with 10% FCS (Life Technologies, Inc., Paisley, United Kingdom) at 37°C in a humidified atmosphere of 5% CO₂ in air. Culture medium, 0.02% EDTA, and trypsin were supplied by the Media Production Unit at the Imperial Cancer Research Fund (Clare Hall, Herts, United Kingdom). Tumor cells were harvested by brief incubation with a 1:3 solution of trypsin/EDTA 0.02%, a single-cell suspension was prepared, and 5 × 10⁶ tumor cells in 0.1 ml of culture medium were injected s.c. into the right flank of the mice. The animals were used for the experiment 17–21 days after tumor inoculation. For the studies of s.c. injections, non-tumor-bearing nude mice were used.

Preparation of Radiolabeled Materials

Pegylated liposome-encapsulated DTPA (Janssen Chimica, Geel, Belgium) was provided by Sequs Pharmaceuticals, Inc. (Menlo Park, CA). STEALTH liposomes are a registered trademark and have been described previously (4). Briefly, 5 ml of DTPA-containing pegylated liposomes [hydrogenated soybean phosphatidylcholine 56.2%, cholesterol 38.3%, N-(carbamoyl)-methoxypolyethylene glycol 2000]-1,2-distearyl-sn-glycero-3-phosphoethanolamine sodium salt 5.3% (values expressed in % molar ratio) were radiolabeled by incubating them with 0.5 ml of 111In-labeled oxine (Amersham International plc, Amersham, United Kingdom) containing 18.5 MBq of radioactivity. After 1 h, 4 mg of EDTA (BDH Ltd, Poole, United Kingdom) was added to chelate any residual unencapsulated 111In and to promote the prompt excretion after administration. Entrapment of 111In within the pegylated liposomes was assayed by loading a 10-µl sample on to 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 gamma counter (Pangbourne, Berks, United Kingdom). Administration proceeded if the entrapment efficiency was found to be >90%.

Uncapsulated ¹¹¹In-DTPA was prepared according to a method described previously (4). Briefly, a 40-µl volume of InCl₃ in 0.04 M HCl containing 22.2 MBq (600 µCi) of radioactivity was titrated to pH 6.0 by the addition of 60 µl of a 3.5% solution of sodium citrate. Thereafter, 10 µl of DTPA, in 10-fold molar excess relative to the InCl₃, and 100 µl of a 100 mM solution of sodium acetate (pH 6.0) were added. The final solution was diluted with PBS to a final activity of 10 µCi/100 µl.

The choice of ¹¹¹In-DTPA as the radioisotope in these studies was based on a number of factors: (a) DTPA reliably and firmly binds ¹¹¹In in vitro and in vivo; (b) DTPA is a small compound with a low molecular weight, similar to that of many of the more commonly used antineoplastic cytotoxic agents; (c) ¹¹¹In has a physical half-life that is sufficiently long to allow detailed analysis of biodistribution of IDLPL and unencapsulated ¹¹¹In-DTPA over the 8-day period of these studies; and (d) a simple and effective means was available of radiolabeling pegylated liposomes with ¹¹¹In-DTPA, which had already been validated in a previous study (4).

Administration of Radiolabeled Materials

Intratumoral Route. Nude mice bearing KB xenograft tumors received an intratumoral injection of 5 µl of either IDLPL or unencapsulated ¹¹¹In-DTPA containing 0.037 MBq (1 µCi) of radioactivity. The injection was performed using a 27-gauge butterfly needle attached via a length of fine tubing to a 100-µl Hamilton microsyringe (Anachem, Luton, Bedfordshire, United Kingdom). After delivery of the injection, the needle was withdrawn slowly from the tumor, and the entry site was observed for evidence of leakage of the injectate through the skin. In the event of observable leakage of injectate from the tumor, the animal was excluded from the study.

Groups of five mice were dissected at 1, 4, 24, 48, 72, and 96 h after intratumoral injection of either IDLPL or unencapsulated ¹¹¹In-DTPA. The mice were anesthetized using inhaled isoflurane (Abbott Laboratories Ltd, Queensborough, Kent, United Kingdom) and killed by exsanguination at cardiac puncture. The aim was to evacuate the maximum blood volume obtainable (≈1.0–1.2 ml). Voided urine was also collected. Thereafter, the tumor, ipsilateral and contralateral inguinal and axillary lymph nodes, liver, spleen, kidneys, and lungs were dissected out, washed in PBS, and placed in preweighed scintillation vials (Sterilin, Stone, United Kingdom). The content of radioactivity was assessed by counting the tubes in the gamma counter with standards of the injected material in triplicate to correct for physical decay of the ¹¹¹In, as described above.

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s.c. Route. Non-tumor-bearing nude mice received a s.c. injection of 100 µl of either IDLPL or unencapsulated ¹¹¹In-DTPA containing 0.37 MBq (10 µCi) of radioactivity in the right flank. The aim was to deliver the injection to an area lying approximately equidistant between the fore and hind limbs. The site of the injection, as identified by the s.c. bleb with a small margin, was then marked with a permanent marker pen to facilitate its localization at the time of dissection.

i.v. Route. Nude mice bearing KB xenograft tumors received an i.v. injection of 100 µl of either IDLPL or unencapsulated ¹¹¹In-DTPA containing 0.37 MBq of radioactivity via a lateral tail vein. Groups of five mice were dissected at 1, 4, 24, 48, 72, and 192 h after s.c. injection of IDLPL and at 15 min and at 1, 4, 24, 48, 72, and 96 h after injection of unencapsulated ¹¹¹In-DTPA. The animals were killed by the same protocol as detailed above. Blood and urine were collected as above. The s.c. injection site, ipsilateral and contralateral inguinal and axillary lymph nodes, liver, spleen, kidneys, and lungs were dissected out, washed in PBS, and placed in preweighed scintillation vials. The content of radioactivity was assessed by counting the tubes in the gamma counter with standards of the injected material in triplicate to correct for physical decay of the ¹¹¹In, as described above.

Groups of five mice were dissected at 1, 4, 24, 48, 72, 96, and 192 h after s.c. injection of IDLPL and at 15 min and at 1, 4, 24, 48, 72, and 96 h after injection of unencapsulated ¹¹¹In-DTPA. The animals were killed by the same protocol as detailed above. Blood and urine were collected as above. The s.c. injection site, ipsilateral and contralateral inguinal and axillary lymph nodes, liver, spleen, kidneys, and lungs were dissected out, washed in PBS, and placed in preweighed scintillation vials. The content of radioactivity was assessed by counting the tubes in the gamma counter with standards of the injected material in triplicate to correct for physical decay of the ¹¹¹In, as described above.

RESULTS

Intratumoral Route. The detailed results for the biodistribution of IDLPL and ¹¹¹In-DTPA after intratumoral injection are presented in Tables 1 and 2, respectively. Fig. 1 illustrates...
the intratumoral levels of radioactivity for both IDLPL and $^{111}$In-DTPA over the period of study. The tumor levels demonstrated prolonged retention of IDLPL over a period of up to 96 h from a maximum level of $76.4 \pm 22.7\%ID/g$ at 1 h to $15.0 \pm 5.5\%ID/g$ at 96 h. These levels compare with the corresponding values for $^{111}$In-DTPA of $66.3 \pm 12.2\%ID/g$ at 1 h and $0.36 \pm 0.15\%ID/g$ at 96 h. The AUC for IDLPL and $^{111}$In-DTPA was 2574.4 and 204.4%ID/gh between 1 and 96 h, respectively. These data represent a 12.6-fold increase in the AUC for the liposome-encapsulated radiolabel in the tumor during the time period of the study. The different rates of clearance of the liposome-encapsulated and -unencapsulated activity are reflected by the levels of radioactivity measured in the blood. At 1 h the level of IDLPL was $0.066 \pm 0.013\%ID/g$, which increased to a peak level of $0.59 \pm 0.42\%ID/g$ at 4 h. Low levels of circulating activity were detectable in the blood until 96 h. The peak measured value in the blood after intratumoral injection of unencapsulated $^{111}$In-DTPA was seen at 1 h at a level of $0.22 \pm 0.02\%ID/g$. No activity was detectable in the blood at $\geq 48$ h. The rapidity of clearance of the unencapsulated radiolabel from the tumor is clearly seen from the profile of urinary excretion of radioactivity with $45.7 \pm 27.5$ and $92.4 \pm 62.4\%ID/g$ present at 1 and 4 h, respectively.

Accumulation of IDLPL was also seen in the local inguinal and axillary lymph nodes over a prolonged period (Fig. 2a). Peak levels were achieved in both the inguinal and axillary nodes at 4 h after the injection. The data confirmed that there was significant drainage and retention of liposomes within the ipsilateral compared with the contralateral lymph nodes. These values showed considerable variability, but, when analyzed according to the ratio of levels in the ipsilateral and contralateral nodal areas, there appeared to be a pattern demonstrating a
progressive increase in the radioactivity in the ipsilateral nodes to a maximum of approximately 8.1 (2.2 versus 0.27%ID/g) for the inguinal region at 24 h and 19:1 (2.5 versus 0.13%ID/g) for the axillary nodes at 48 h. In comparison, the same data for unencapsulated $^{111}$In-DTPA showed no evidence of progressive accumulation in regional nodes, no evidence of increased uptake in the ipsilateral compared with the contralateral lymph node groups, and no suggestion of prolonged retention (Fig. 2b).

The data for the major organs (liver, spleen, kidneys, and lungs) showed low levels of uptake of IDLPL over a prolonged period. There was evidence of accumulation of radioactivity in the liver, spleen, and kidneys, consistent with progressive deposition in these tissues, because the liposomes were gradually released from the tumor into the circulation. The lungs showed no evidence of progressive accumulation but, rather, conformed to the pattern seen for blood radioactivity. The levels of uptake of $^{111}$In-DTPA in the major organs were very low, with no evidence of progressive accumulation or retention.

**s.c. Route.** The detailed results for the biodistribution of IDLPL and $^{111}$In-DTPA after s.c. injection are shown in Tables 3 and 4, respectively. Figure 3 illustrates the s.c. levels of radioactivity for both IDLPL and $^{111}$In-DTPA over the period of study. These data clearly demonstrate that the IDLPL was cleared very slowly from the injection site with 74.7 ± 9.5% ID/g retained at 192 h. These data contrast directly with those for unencapsulated $^{111}$In-DTPA, which was cleared rapidly from the s.c. injection site from a maximal level of 59.1 ± 13.7%ID/g at 15 min to 3.7 ± 1.0%ID/g at 1 h. The corresponding AUC between 1 and 192 h for IDLPL and $^{111}$In-DTPA were 24,051 ± 46.4%ID/g/h, respectively. These data represent a 518-fold increase in the AUC for the liposome-encapsulated radiolabel at the s.c. injection site in this time period. The levels of radioactivity measured in the blood showed evidence of these different patterns of absorption. The peak blood level of IDLPL of 0.74 ± 0.09%ID/g was reached at 24 h, whereas for the unencapsulated $^{111}$In-DTPA, the maximum level of 3.2 ± 0.3%ID/g was measured in the blood at 15 min, falling rapidly to 0.27 ± 0.04%ID/g at 1 h.

Significant levels of IDLPL were detected in the ipsilateral inguinal and axillary lymph nodes for prolonged periods after s.c. injection (Fig. 4). When compared with the corresponding contralateral nodal groups, maximal ratios of 121:1 (57.9 versus 48.4%ID/g) at 24 h for the inguinal nodes and 17:1 (5.2 versus 3.0%ID/g) at 72 h for the axillary nodes were documented.
injected dose per gram (± SD).

and beyond.

in any of the nodes, with levels essentially undetectable at 24 h in any of the nodes, with levels essentially undetectable at 24 h. There was no evidence of accumulation of this radiolabel intratumoral and the s.c. injections. For unencapsulated 111 In-DTPA in tumor, inguinal, and axillary nodes after i.v. injection (31). As yet, there have been no studies of direct evidence of the ability of the agents retained in them to become radiolabeled in the liver, spleen, and bones. These data contrast with those recorded for unencapsulated pegylated liposomes.

The results for the levels of IDLPL and 111 In-DTPA after s.c. injection in nude mice. Data expressed as mean % injected dose per gram (± SD).

**DISCUSSION**

Direct locoregional drug administration has an obvious appeal in the treatment of cancer in that it immediately achieves a high drug concentration at the desired site of action and avoids the need for initial systemic administration with all of the associated adverse effects. Intratumoral injection represents the most direct form of such treatment but, as yet, has failed to establish a role in the standard treatment of any solid cancer. This apparent paradox can be explained as being largely attributable to the following factors: (a) rapid drug clearance from the tumor interstitium; (b) dose-limiting direct normal-tissue toxicity arising from local drug diffusion; (c) normal-tissue toxicity caused by systemic absorption; and (d) efficacy of surgical excision or irradiation for lesions that are accessible to intratumoral injection. Attempts have been made to overcome the first three problems. Reduced clearance from the site of injection and attenuated local normal tissue toxicity can be achieved by immobilizing the drug in a form that binds to a local receptor (22–24) or by preparing it in a sustained-release macromolecular form that is too large to diffuse away rapidly (25, 26).

The data presented here have demonstrated that pegylated liposomes can keep the entrapped agent at the tumor site with a 12.6-fold increase in the AUC within the tumor for IDLPL relative to 111 In-DTPA. Inspection of Fig. 1 shows that, had dissection time points been performed beyond 96 h, the calculated difference between the AUC for encapsulated and unencapsulated 111 In-DTPA would have been considerably higher. In the context of targeted delivery of radiosensitizers, the prolonged retention of IDLPL that is documented here suggests that they may act as an effective means of achieving sustained intratumor release of entrapped agents. As regards the issue of local normal tissue toxicity, Madhavan and Northfelt (10) have reported that encapsulation of doxorubicin within pegylated liposomes abrogates the severe local toxicity of this agent after inadvertent extravasation (27, 28). It is likely that encapsulation of other radiosensitizing agents, such as cisplatin or 5-iodo-2'-deoxyuridine, would afford similar protection against local toxicity. In addition, the systemic toxicity arising from the absorption of pegylated liposomal agents might reasonably be expected to be reduced, in line with the data for i.v. administration (6–9).

Therefore, on this basis, pegylated liposomes seem to be an attractive vehicle for the delivery of locoregional therapy.

The above considerations will only have clinical utility, however, if the contents of locally administered pegylated liposomes are released within the interstitium of the tumor. In this regard, there is compelling evidence confirming such release in tumor tissue after i.v. administration. In particular, microfluorimetric techniques have shown release of doxorubicin within xenograft tumor deposits after the initial accumulation of liposomes in the perivascular space (29, 30). Furthermore, the responses of a variety of xenograft tumors to therapeutic nonpegylated and pegylated liposomes provides a wealth of indirect evidence of the ability of the agents retained in them to become bioavailable and exert a biological effect (5). The superiority of both pegylated liposomal doxorubicin and cisplatin over the respective unencapsulated agents in this tumor model also has been confirmed in studies in which the agent was delivered by i.v. injection (31). As yet, there have been no studies of direct implications of pegylation for this route of administration.
intratumor injection of liposomal therapeutic agents. Konno et al. (32) reported a significant reduction in the growth rate of s.c. AH-66 hepatoma tumors with little toxicity after peritumoral s.c. injections of interleukin-2 encapsulated in nonpegylated, small unilamellar vesicles.

The data from these studies of intratumoral administration suggest that this approach also may represent an effective means of targeting the locoregional lymph node drainage areas, because substances injected directly into tumor deposits may be cleared from the tumor, at least in part, via lymphatic channels in a pattern that may recapitulate the likely spread of lymphatic metastases. The validity of this approach has been supported in recent years by studies seeking to identify “sentinel nodes” in patients with breast cancer and malignant melanoma by intratumoral injections of radiolabeled colloids (33, 34). Therefore, in addition to local therapeutic effects in the tumor, direct intratumoral injection of pegylated liposomal agents may achieve the additional benefit of concentrating them within the sites of lymphatic spread over a prolonged period, without causing unacceptable local toxicity. Although the absolute levels of radiolabeled liposomes that accumulated in the locoregional nodes after intratumoral injection were relatively low, the pattern of preferential deposition in ipsilateral as opposed to contralateral nodes confirmed that there was locoregional trafficking of pegylated liposomes within the lymphatic system. The retention of the radioactivity within the nodal tissue confirmed that it remained encapsulated within a liposome; otherwise, it would have been cleared as rapidly as unencapsulated $^{111}$In-DTPA, which showed no evidence of prolonged nodal deposition. However, the relatively poorly developed lymphatic drainage of the tumors (as shown by the relatively slow clearance of $^{111}$In-DTPA after intratumoral but not s.c. injection) and the absence of lymph node metastases from KB xenograft tumors (data not shown) suggest that this model may not accurately reflect clinical situations. For SCCHN, the patterns of lymphatic metastasis occur in a predictable fashion based on
Groups of five animals were dissected at 1, 24, 48, 72, 96 and 192 h after i.v. injection of 0.037 MBq of IDLPL, and the tissue content of radioactivity was assessed by counting samples in a gamma counter. Data are expressed as mean % injected dose per gram ± SD.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>192 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>61.8 ± 24.6</td>
<td>8.4 ± 3.6</td>
<td>6.4 ± 3.5</td>
<td>4.3 ± 3.0</td>
<td>5.9 ± 2.4</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>Blood</td>
<td>34.6 ± 3.1</td>
<td>22.4 ± 2.5</td>
<td>6.4 ± 2.2</td>
<td>0.32 ± 0.12</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Tumor</td>
<td>1.4 ± 0.4</td>
<td>5.0 ± 2.7</td>
<td>5.1 ± 2.4</td>
<td>3.2 ± 1.5</td>
<td>1.8 ± 0.9</td>
<td>0.85 ± 0.36</td>
</tr>
<tr>
<td>IILN*</td>
<td>1.3 ± 0.2</td>
<td>3.4 ± 1.2</td>
<td>2.2 ± 0.8</td>
<td>3.8 ± 0.6</td>
<td>2.4 ± 1.0</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>CILN</td>
<td>1.3 ± 0.2</td>
<td>3.0 ± 1.0</td>
<td>2.6 ± 0.5</td>
<td>3.6 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>IALN</td>
<td>1.9 ± 0.3</td>
<td>2.8 ± 0.6</td>
<td>2.9 ± 0.9</td>
<td>2.8 ± 0.4</td>
<td>2.0 ± 0.7</td>
<td>0.85 ± 0.42</td>
</tr>
<tr>
<td>CALN</td>
<td>1.7 ± 0.5</td>
<td>2.7 ± 0.8</td>
<td>2.7 ± 0.9</td>
<td>2.7 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>5.4 ± 1.8</td>
<td>18.1 ± 3.2</td>
<td>16.9 ± 2.9</td>
<td>16.1 ± 3.4</td>
<td>13.5 ± 2.6</td>
<td>5.7 ± 2.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.4 ± 2.0</td>
<td>19.1 ± 2.6</td>
<td>15.6 ± 3.4</td>
<td>14.4 ± 2.9</td>
<td>11.6 ± 3.2</td>
<td>7.5 ± 2.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.5 ± 2.4</td>
<td>6.0 ± 2.6</td>
<td>5.4 ± 1.8</td>
<td>5.1 ± 1.7</td>
<td>4.0 ± 1.7</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>Lung</td>
<td>6.8 ± 3.0</td>
<td>2.4 ± 1.3</td>
<td>1.5 ± 0.8</td>
<td>0.73 ± 0.28</td>
<td>0.34 ± 0.15</td>
<td>0.20 ± 0.07</td>
</tr>
</tbody>
</table>

* IILN, ipsilateral inguinal lymph node; IALN, ipsilateral axillary lymph node; CILN, contralateral inguinal lymph node; CALN, contralateral axillary lymph node.

The s.c. space has not been evaluated in detail as a potential route of clinical administration for cytotoxic therapies, although it is used for the delivery of cytokines (IFN, interleukin 2; Ref. 37), luteinizing-hormone-releasing hormone analogues (38), and a range of agents in the palliative care setting (39). A number of preclinical studies of s.c. administered nonpegylated liposomal agents as depot preparations have been reported in the context of inflammatory/infective (40–42) and neoplastic (43, 44) disorders. Because the s.c. space itself is not a meaningful target for anticancer therapies, the use of s.c. injected pegylated liposomes is likely to be valuable only in the context of locoregionally targeted therapeutic strategies. The s.c. tissues are permeated by a rich network of lymphatic channels that drain to locoregional lymph nodes. These nodes also receive afferent lymphatic channels from primary tumor masses. Therefore, the administration of pegylated liposomal agents by the s.c. route may provide a useful means of delivering high concentrations of drugs to lymph nodes clinically involved with metastatic disease or, indeed, clinically uninvolved lymph nodes that may harbor micrometastatic disease. Kaledin et al. (45) demonstrated the ability of s.c. injections of liposomal cisplatin and hydrocortisone to reduce the incidence of regional (ppliteal) lymphadenopathy after injection of murine hepatoma and pulmonary adenocarcinomas into the footpad of mice. Significantly, there was no effect on the incidence of distant metastatic disease, suggesting that the liposomal therapy was exerting only a locoregional effect. Effective lymphatic targeting of 125I-Radiolabeled pegylated liposomes to target the cervical and axillary nodes after s.c. injection in a rodent model. Interestingly, with their formulation of pegylated liposomes of 80- to 90-nm diameter, up to 30% of the injected radiolabel was
Intratumoral and s.c. Pegylated Liposomes

detected in the blood between 12 and 24 h after s.c. injection. Such levels are greatly in excess of the levels reported here, which suggests that this formulation possesses a greater ability to act as a locoregional depot agent.

The studies reported here provided clear evidence of drainage of pegylated liposomes to locoregional lymph nodes. The levels achieved in the ipsilateral inguinal lymph nodes were significantly higher after s.c. injection compared with i.v. injection. That the measured radioactivity within the lymph nodes was likely to be retained within liposomes was strongly suggested by the fact that there was no evidence of the retention of unencapsulated $^{111}$In-DTPA within the locoregional lymph nodes. Therefore, it is likely that in the setting of lymph nodes involved with metastatic disease, the tumor deposits would be exposed to relatively high concentrations of the encapsulated drug. In the context of the treatment of SCCCHN, this approach may be useful as a means of targeting radiation sensitizers to lymph nodes containing clinically apparent deposits of metastatic disease or even nodal areas suspected of harboring micrometastatic disease. The patterns of lymphatic drainage of the skin in the head and neck are well documented. Thus, it may be possible to inject pegylated liposomes containing radiosensitizers s.c. in an area that will drain to lymph nodes that will be included in the radiation treatment portals. If the site of the s.c. injection lies beyond the field boundaries of the radiation portal, this area will not be sensitized to the effect of the radiation, and there should be little or no additional toxicity from this strategy. However, it must be borne in mind that the s.c. space of a loose-skinned animal such as a mouse is very different from that of humans. The capacity of the s.c. space in mice is large, such that it will accommodate relatively large volumes of injectate. In contrast, the s.c. space in humans is a potential space with limited capacity, especially in areas such as the head and neck. Attempts to deliver large injection volumes in these sites is likely to be limited by pain. Nonetheless, small-volume, single or repeated injections should be feasible without excessive toxicity.

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Pegylated Liposomes Have Potential as Vehicles for Intratumoral and Subcutaneous Drug Delivery

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