Tissue Dosimetry of Liposome-Radionuclide Complexes for Internal Radiotherapy: Toward Liposome-Targeted Therapeutic Radiopharmaceuticals

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Abstract. Background: Quantitative examination of the important physical parameters, such as the tumor absorbed dose and the tumor-to-normal-tissue (T-N-T) absorbed dose ratios, for effective use of radionuclide-liposome conjugates in internal radiotherapy was carried out. Methods: The Medical Internal Radiation Dose (MIRD) formalism was used to develop a set of dosimetric equations. Pharmacokinetic functions used as input information to the dosimetric model were derived from experimental time-biodistribution data. Multilamellar (MLV), small unilamellar (SUV) and sterically stabilized (GM1- and PEG-coated) liposomes were examined in combination with the very promising particle emitting radionuclides: ⁶⁷Cu, ¹⁸⁸Re and ⁴⁴⁷At. For comparative purposes, the widely used, ⁹⁰Y and ⁱ³¹I were also included in the study. For all radionuclide-liposome combinations, the mean absorbed dose per amount of radioactivity administered was obtained: (i) for two different types of human xenografts located in the muscle and liver tissue, and (ii) for normal liver, spleen, kidneys, and total body. Results: Regardless of radionuclide, the poorest values were obtained for the MLV liposomes. Due to more rapid uptake of sterically stabilized (GM-coated) liposomes to the muscle tumor tissue as compared to SUVs, ⁴⁴⁷At and ¹⁸⁸Re deliver higher tumor doses when combined with the former, while ⁶⁷Cu, ⁹⁰Y and ⁱ³¹I are more effective with SUVs. The most promising results were obtained for the [⁴⁴⁷At-GM] complex in the liver tumor. Conclusion: The importance of liposome size and steric barrier when designing effective radionuclide-carrier systems was revealed, but most importantly the optimal matching between the radionuclide half-life and the time of maximum liposome accumulation ratio between the tumor and normal tissue.

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Key Words: Radiotherapy, liposome, radionuclide, tumor targeting, dosimetry, radiopharmaceuticals.

0250-7005/2000 $2.00+.40
approximation of radiation doses to humans by various combinations of radionuclide-liposome conjugates. Dosimeteric considerations are pivotal in the evaluation of any novel radiotherapeutic modality, due to the need to obtain optimum tumor-to-normal-tissue radiation energy deposition, thus crucial in directing the optimum selection of the liposome system and radionuclide towards the formulation of radiopharmaceuticals. It is expected that the acquired knowledge obtained herein, along with the thoroughly studied radiolabeled monoclonal antibodies (radioimmuno-therapy) will help to construct effective modalities with improved therapeutic response ratios.

Materials and Methods

Liposome systems. All types of liposome systems were included in the present study (MLV, SUV, GM1- and PEG-coated sterically stabilized liposomes); biodistribution data for the multilamellar vesicles (MLV) and the small unilamellar vesicles (SUV) composed of distearoylphosphatidylcholine (DSPC), cholesterol (cholesterol) and dicetylphosphate (DCP) (10:5:1) were obtained from the work of Ogihara et al. (18). While biodistribution data for the sterically stabilized liposomes prepared from DSPC, Chol and monosialoganglioside (GMI) (2:1:0.2) and hydroxylated PC, cholesterol and PEG-DSPE (10:7.1) were taken from Huang et al. (19). These were found to be the most complete analytic biodistribution studies published to date on tumor-bearing mice, where sufficiently detailed time-distribution data (at least four time points per curve) in various organs were presented. For all liposome systems examined, tumor, liver, spleen and (normal) total-body were the critical tissues considered. Two model tumors were implanted in the biodistribution studies mentioned above: an Ehlich solid tumor subcutaneously injected in the hind leg (18); and a C-26 colon carcinoma inoculated either into the liver or subcutaneously injected into the flank (19). Both experimental model tumors were of small size, being approximately 0.5 g weight and 5 mm diameter.

Radionuclides. The rationale for selecting the optimum radionuclide catalysts considerations about two physical parameters; namely: its physical characteristics or late dose, and its chemical stability. Since there is limited control over the biological half-life of the carrier molecule judicious selection of the radionuclide's decay rate is warranted for optimum effective half-life. As has been shown for other targeting agents, for example, significant improvement of therapeutic efficacy is achieved by the use of longer-lived radionuclides when the tumor uptake is prolonged in comparison to critical organs (20).

Along with the decay rate the emitted radiation needs also to be optimized according to the geometry of the tumor. In contrast to imaging applications, radionuclides exhibiting high emission fraction of short range particulate radiation, namely, low energy electrons and a-particles, are needed. Such particles are capable of depositing cytotoxic amounts of radiant energy along their track having a sparing effect on normal tissues. Nonetheless, a moderate emission of electromagnetic radiation (X- or y-rays) is desirable for imaging their biodistribution by trace amounts prior to therapy. For these reasons, radionuclides of considerably different half-lives and emission properties were examined (see Table 1). Although the therapeutic use of 67Cu, 186Re and 211At is still mainly at the experimental level they are recognized as offering very promising characteristics for targeted radiotherapy and are expected to be part of the new generation of internally administered radiotherapeutics (21, 22). Their performance was also compared with the widely used 123I and 131I.

Table 1. Radiation emission properties of radionuclides.

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>1/2 (hours)</th>
<th>Eγ (MeV)</th>
<th>Range in tissue (mm)</th>
<th>Eγ (Kev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>131I</td>
<td>192</td>
<td>0.182 (β)</td>
<td>0.9</td>
<td>3.64 (81 %)</td>
</tr>
<tr>
<td>90Y</td>
<td>64</td>
<td>0.935 (β)</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>67Cu</td>
<td>62</td>
<td>0.141 (β)</td>
<td>0.7</td>
<td>185 (49 %)</td>
</tr>
<tr>
<td>186Re</td>
<td>17</td>
<td>0.764 (β)</td>
<td>3.5</td>
<td>155 (15 %)</td>
</tr>
<tr>
<td>211At</td>
<td>7</td>
<td>6.8 (α)</td>
<td>0.06</td>
<td>77-92 (58 %)</td>
</tr>
</tbody>
</table>

Results

The radiation dose deposited in the tumor tissue per amount of radioactivity injected by the different liposome systems is depicted in Figure 1. Evident from this figure, is the many-fold higher radiation doses delivered to the tumor when 211At was used compared to the other nuclides (except the 90Y-SUV conjugate), despite the fact that the total number of 211At decays inside the tumor volume were fewer due to its shorter half-life. This is mainly because of the emission properties of 211At exhibiting the advantageous potential that lies in delivering α-particle emitters to tumor tissues. Effective targeting is more crucial in this case, since much higher levels of energy are
Tumor Radiation Absorbed Dose
(mGy/mCi)

Figure 1. Radiation absorbed dose in the muscle and liver tumor from various radiolabeled conjugates of MLV, SUV, PEG, and GM1 coated liposomes.

The data shows that MLV, SUV, GM1, PEG, and GM1* each have different radiation dosages in muscle and liver tumors. The x-axis represents different radionuclides: Cu-67, Re-188, At-211, Y-90, and I-131.

Deposited in the tissues (in the MeV range, see Table I). Interestingly enough, 90Y also deposited high amounts of energy in the tumor tissue, particularly when compared to 131I, which are both commonly used in the clinic today. When 90Y was combined with SUV, the amount of energy deposited in the tumor was the highest of all liposome-radionuclide combinations, exemplifying the importance of both half-life and emission properties in selecting the optimum radionuclide for a particular liposome system.

When looking at the liposome systems, MLVs were rapidly and efficiently cleared from circulation to the liver and spleen, as previously described (30), thus depositing minimal amounts of radiation (3-10 fold lower doses than the rest of liposome systems). Also, the difference in the total amount of radiant energy deposited in the tumor mass between the two sterically stabilized systems was remarkable. This result indicated the dramatic effect that the coating on the liposome surface can have on the overall therapeutic effectiveness of the carrier. In the case of the tumor model located in the liver, the radiation doses delivered by the GM1-sterically stabilized liposomes were higher (asterisk) for all radionuclides studied compared to the muscle tumor model (Figure 1). This result was obtained since liver is a critical organ exhibiting high liposome accumulation. Thus tumors located in the liver tissue are expected to have high liposome concentration and concomitantly absorb higher amounts of radiant energy, both from radionuclides decaying inside the tumor volume as well as from decays occurring in the surrounding liver tissue (the magnitude of the latter effect obviously depends on the amount of penetrating radiation emitted by the radionuclide).

The most crucial observation from Figure 1 is that, for the short half-life radionuclides ($^{186}$Re and $^{211}$At, 17 and 7 hours, respectively) GM1-coated liposomes delivered higher radiation doses in muscle tumor than the SUV, due to their faster tumor accumulation. The opposite was true for the long half-life radionuclides ($^{64}$Cu, $^{90}$Y and $^{131}$I; 3, 3 and 8 days, respectively). This indicates that efficient deposition of liposome-mediated radiant energy depends both on the emission properties of the radionuclide and the resonance of its decay rate with the biodistribution performance of the liposome system.

Table II depicts the tumor-to-organ radiation absorbed dose ratio for liver and spleen, since these are the normal tissues with the highest liposome concentration per organ mass. The importance of liposome size in the tumor-to-organ analysis is evident, whereby the shift from the large MLV structures (>1 μm) to the small SUVs (<100 nm mean diameter) significantly improved the T-N:NT ratios. The spleen exhibiting the lowest T-NT ratios was the most critical organ in all cases (except for MLVs where the liver seemed to compete with the spleen). The results also indicated that the application of GMI-coated liposomes to
Table II. Tumor-to-normal-tissue radiation absorbed dose ratio.

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</tr>
</thead>
<tbody>
<tr>
<td>MLV</td>
<td>0.71</td>
<td>0.35</td>
<td>0.92</td>
<td>0.44</td>
<td>1.15</td>
<td>0.59</td>
<td>0.67</td>
<td>0.35</td>
<td>0.80</td>
<td>0.35</td>
</tr>
<tr>
<td>SUV</td>
<td>0.76</td>
<td>0.48</td>
<td>0.81</td>
<td>0.55</td>
<td>0.80</td>
<td>0.54</td>
<td>0.75</td>
<td>0.48</td>
<td>0.84</td>
<td>0.50</td>
</tr>
<tr>
<td>GMI</td>
<td>0.90</td>
<td>0.70</td>
<td>1.11</td>
<td>0.76</td>
<td>1.23</td>
<td>0.84</td>
<td>1.12</td>
<td>0.74</td>
<td>1.13</td>
<td>0.67</td>
</tr>
<tr>
<td>PEG</td>
<td>0.47</td>
<td>-</td>
<td>0.46</td>
<td>-</td>
<td>0.44</td>
<td>-</td>
<td>0.50</td>
<td>-</td>
<td>0.46</td>
<td>-</td>
</tr>
<tr>
<td>GMIP</td>
<td>0.90</td>
<td>0.70</td>
<td>1.11</td>
<td>0.76</td>
<td>1.23</td>
<td>0.84</td>
<td>1.12</td>
<td>0.74</td>
<td>1.13</td>
<td>0.67</td>
</tr>
</tbody>
</table>

T: Tumor; S: Spleen; LV: Liver
(*) refers to the liver tumor model; otherwise muscle tumor model

The liver tumor model was most promising since the highest T:NT values were found in this case. It has to be noted though, that conjugates that may deposit high doses of radiation in the tumor tissue (from Figure 1) can exhibit low T:NT values and therefore be detrimental for therapeutic applications. An example of that is the [90Y-SUV] conjugate, which deposited the highest amount of energy to the tumor than any other radiolabeled SUV (Figure 1), but its T:NT ratios (particularly the tumor-to-spleen) (Table II) were too low for therapeutic applications.

In Figure 2, the tumor-to-non-tumor (normal total-body) radiation absorbed dose ratios are represented for all radionuclides studied and all liposome systems. Generally all liposome systems (apart from MLV) delivered efficient radiation doses to tumors relative to the total radiation body-burden they induced. The GMI-coated liposomes in the tumor xenograft located in the liver manifested the best values. However, PEG-coated liposomes were evidently much worse at least in the present case of muscle tumor, irrespective of the conjugated radionuclide. Also, between the most widely used β-particle emitting nuclides, 131I and 90Y, 131I-liposome conjugation resulted in the lowest ratios for all liposome systems (except MLV) and tumor models studied, which was partly expected on the basis of its higher penetrating radiation component. Interestingly, when 186Re was conjugated with almost any liposome system, the tumor-to-total-body ratio obtained was the highest, thus 186Re was the most favourable from a radiation risk perspective.

Discussion

The most critical physical parameter for the purposes of internal radiotherapy is the tumor radiation absorbed dose which eventually determines the tumoricidal effect of any therapeutic modality. The tumor-to-normal-tissues (T:NT) ratios, however, commonly indicate the therapeutic limitations posed by the maximum tolerance levels of normal tissues. In the present study, it has been clearly illustrated that correlation between the radionuclide half-lives and the particular time-biodistribution curves of each liposome system was primarily responsible for the considerably different radiation doses delivered by the various liposome-nuclide conjugates.

From the analysis offered herein, the magnitude of the radiation dose delivered to the tumor tissues was comparable to that offered by some very promising radiolabelled MoAbs (31, 32). Considering that conventional external beam radiotherapy commonly utilizes fractions of about 1-2 Gy in the tumor (per day over a period of a few weeks) it is apparent that, for most of the conjugate systems depicted in Figure 1, a comparable radiation dose to the tumor with external beam radiotherapy may be achieved by repeated administrations of about 100 mCi or less (resulting in 1-10 Gy in the tumor depending on the conjugate system used).

Also, there are evident advantages in delivering α-particle emitters via liposomes, as shown in the case of 211At-GMI conjugate, provided that efficient targeting and diffusion in the tumor is achieved. This is attributed to the nature of the α-particle radiation emitted from the 211At nucleus whereby 6.8 MeV of energy is imparted entirely within a very small volume (of about 60 μm radius) from its origin. This is in contrast to the other radionuclides examined, which decay by the emission of β-particles. The latter, though of lower energy (in the KeV region), possess a much larger path length (in the mm range). Thus, in the case of 211At, both larger amounts of energy are emitted per nuclide decay and also the emitted energy is absorbed in a much smaller volume, further enhancing the radiation dose delivery capabilities of that nuclide.

The quantitative insight in the time-distribution of tissue
radiation absorbed doses, as presented in this study, is also of significant clinical importance when adjuvant therapeutic modalities are employed. Such adjuvant therapies may aim towards (i) altering the target tissue (tumor in this case) radiosensitivity or radioresistance; examples are pre-injections with compounds such as muramyl dipeptide to induce production of cytokines enhancing organ/tissue radioprotection (33, 34); (ii) elevating the barriers to penetration, movement and perfusion of therapeutic molecules or targeting delivery systems inside the tumor tissue, posed by the tumor physiology (35, 36); examples are the pre-injection with compounds such as peptide SP which increases vascular permeability in tissues expressing SP receptors (37), or the local application of hyperthermia (38); (iii) altering the therapeutic molecules’ or vehicles’ biodistribution; as for example by pre-injection with RES suppression or saturation agents (39, 40); and (iv) removing the radiolabeled liposomes from the circulation at the time point of maximum tumor accumulation by specific biotin-avidin interactions, as used to optimise tumor imaging (41, 42). Since the effects of the adjuvant therapeutic modalities mentioned above are transient, knowledge of the time distribution of deposited radiative energy in the critical tissues by the liposome-radionuclide conjugates will determine their clinical effectiveness. Similar approaches have been also evaluated in the case of internal radiotherapy by using radiolabeled monoclonal antibodies (MoAb) (43, 44).

Liposomes have been almost totally overlooked as carriers of particle emitting radionuclides for therapeutic purposes. The present study showed that for internal radiotherapeutic applications sterically stabilized liposomes may not be the most preferred system (e.g. PEG-coated liposomes). Contrary to chemotherapy where enhanced blood circulation half lives lead to improved therapeutic effect, in radiotherapy more complex parameters will determine optimum therapeutic index. Moreover, radionuclide-carrying long-circulating liposomes may cause enhanced damaging effects to the blood and the epithelial cells lining blood vessels. Nevertheless, the present study indicated that liposomes can be useful delivery vehicles of radionuclides for internal radiotherapy applications as long as an effective matching between the radionuclide half-life and the time of maximum liposome accumulation ratio between the tumor tissue and normal organs is achieved.

Conclusion

Based on animal time-biodistribution data and a well-established dosimetric formalism we have provided a first approximation of radiation doses to humans for a variety of radionuclide-liposome conjugates. This is considered
essential for a prudent pre-clinical evaluation of the proposed radiotherapeutic modality and in determining starting dose levels and dose increments for a Phase I study. As also found with other targeting agents, matching the radionuclide’s half-life with liposome time-biodistribution is critical in selecting the optimum conjugate. Certainly, experimental data with regard to liposome microdistribution within tumors are greatly needed to properly assess the effectiveness of the different emission properties of the radionuclides (particularly in the case of α-emitters). Also, further investigation is required in order to construct liposome-radionuclide conjugates which with the attachment of targeting ligands (such as MoAb), may utilize the advantages of radioimmunotherapy with the capabilities offered by liposome systems towards novel therapeutic radiopharmaceuticals.

References


Kostarelos and Emfictzoglou: Liposome-Mediated Radionuclide Tumor Targeting


Received May 2, 2000
Accepted July 7, 2000