Pegylated Liposome-encapsulated Doxorubicin and Cisplatin Enhance the Effect of Radiotherapy in a Tumor Xenograft Model

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ABSTRACT

Concomitant chemotherapy and radiotherapy (CCRT) has recently been shown to improve treatment outcome in a range of solid tumors. Pegylated liposomes have the potential to target drugs directly to tumors and may increase the efficacy and reduce the toxicity of CCRT by selectively delivering radiosensitizing agents to tumor, as opposed to normal, tissues. In these studies, we have assessed CCRT using pegylated liposome encapsulated doxorubicin (PLED) and pegylated liposome encapsulated cisplatin (PLEC) against KB head and neck cancer xenograft tumors in nude mice. The addition of low-dose (2 mg/kg) PLED (P < 0.001) and PLEC (P < 0.001) significantly increased the effect of 4.5 Gy, but not 9 Gy, single-fraction radiotherapy (SFRT). Both PLED and PLEC were significantly more effective than their unencapsulated counterparts in increasing the effect of SFRT. In addition, PLED (P < 0.001) and PLEC (P < 0.05) significantly increased the effect of fractionated radiotherapy (9 Gy in 3 fractions) in two different dosing schedules (2 mg/kg single dose or three sequential doses of 0.67 mg/kg). Unencapsulated diethylenetriaminepentaacetic acid and pegylated liposomal diethylenetriaminepentaacetic acid were used as controls to test the effect of the liposome vehicle and showed no interaction with 4.5 Gy or 9 Gy SFRT (P > 0.1). CCRT was well-tolerated, with no evidence of increased local or systemic toxicity, as compared with radiotherapy alone. This study is the first to demonstrate the value of pegylated liposomes as vehicles for the delivery of radiosensitizing drugs in CCRT strategies.

INTRODUCTION

Two recent meta-analyses have provided encouraging data regarding the use of CCRT in the treatment of SCCHN (1, 2). As a consequence, considerable research effort is currently being devoted to the development of CCRT strategies (3, 4), but there are potential obstacles to combining these two treatment modalities. RT and cytotoxic chemotherapy frequently share overlapping profiles of normal local tissue toxicity, such as acute mucosal and cutaneous toxicity. As a result, patients may develop an exaggerated mucosal reaction to the combined therapy with the need for radiation dose reductions and treatment delays, both of which are associated with reduced local control (5). In addition, there is some evidence that CCRT is associated with an increase in late local radiation morbidity (6). Furthermore, cytotoxic agents such as cisplatin, 5-fluorouracil, paclitaxel, methotrexate, and bleomycin, which are active against SCCHN (reviewed in Ref. 7) have appreciable patterns of systemic toxicity. Therefore, delivering these agents to patients with SCCHN, who often have coexisting medical conditions associated with tobacco and alcohol consumption, can be associated with considerable morbidity.

Encapsulation of cytotoxic drugs within a pegylated liposomal matrix may circumvent some of the limitations of CCRT. Liposome encapsulation may enhance localization of the drug within tumor deposits by virtue of the relative increase in vascular permeability of tumor neovascularization as compared with adjacent dose-limiting normal tissues. This effect would tend to increase the drug concentration and the area under the concentration/time curve at the therapeutic site. The limited data available for pegylated liposomes from preclinical and clinical studies have confirmed selective delivery of liposomes to tumor deposits and support this approach (8–11). Although there are no definitive data on the time course of liposome clearance from tumor tissues, there is evidence that they release their contents over a prolonged period. Therefore, once they have localized to the tumor, they have the ability to act as a depot preparation for sustained intratumoral drug release (11). This phenomenon may be particularly beneficial during a course of daily RT because each fraction would be delivered while the drug was present in

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2 The abbreviations used are: CCRT, concomitant chemotherapy and radiotherapy; SCCHN, squamous cell cancer of the head and neck; RT, radiotherapy; FRT, fractionated radiotherapy; PLED, pegylated liposome encapsulated doxorubicin; PLEC, pegylated liposome encapsulated cisplatin; DOX, doxorubicin; Vo, initial volume; CDDP, cisplatin; DTPA, diethylenetriaminepentaacetic acid; TLD, thermoluminescent dosimeter; SFRT, single-fraction radiotherapy.
the tumor without the need for daily drug dosing. In addition to the benefits of selective tumor deposition, liposome encapsulation has been shown to reduce significantly the systemic toxicities of a range of agents (reviewed in Ref. 12). This effect would tend to increase the tolerability of CCRT and, perhaps, facilitate escalation of the drug dose. This may additionally increase tumor drug localization and improve the therapeutic ratio.

This paper describes studies carried out using combined drug and radiation treatment in mice bearing KB xenograft tumors. Preparations of PLED and PLEC were studied. Each of these agents has documented activity against SCCHN (13–16), and both drugs are known to enhance the effects of ionizing radiation on tumor cells (17, 18). The precise nature of the interaction (additive or supraadditive) between these drugs and radiation is difficult to discern in vivo (5), although such agents are frequently described as “radiosensitizers.” In these studies, we followed that practice without meaning to draw any firm conclusions about the mechanism of the interaction.

Here we demonstrate for the first time the efficacy of doxorubicin and cisplatin in pegylated liposomes delivered as a combined approach with RT against tumor xenografts and describe investigations into the most appropriate scheduling of this combined approach.

MATERIALS AND METHODS

Cell Line. Human SCCHN KB tumor cells were grown in RPMI 1640 medium 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.

Tumor Model. KB cells were harvested by brief incubation with a 1:3 solution of trypsin/versene (EDTA 0.02%), and a single-cell suspension was prepared. Xenograft tumors were established by injecting 5 × 10⁶ tumor cells in 100 μl of culture medium without FCS s.c. into the right flank of nude mice. The animals were used for experiment at 14 days, at which time tumors of ~8 mm in diameter were present.

Assessment of Tumor Growth. Starting 7 days after inoculation, the tumors were measured on at least three occasions before the start of treatment. Three orthogonal diameters [length, breadth, and height (d₁, d₂, and d₃)] were recorded using Vernier calipers. The tumor diameter was calculated using the formula: 

\[ V = \frac{4}{3}\pi d_1 d_2 d_3 \]

The tumor volume on the day of tumor RT was designated as the initial volume, or Vo. Tumor volume was assessed two or three times/week, and the absolute and relative (as compared with Vo) tumor volumes were calculated. Mice were killed after the tumor had increased in size to more than three times its original volume (3Vo). The time taken to reach 3Vo was recorded and used as a surrogate measure of animal survival on the assumption that those tumors which had tripled their original volume were destined to increase in size inexorably. Use of this measure was designed to spare the animals from the physical distress of unnecessarily large tumor burdens and to comply with the Medical Research Council guidelines (Responsibility in the Use of Animals for Medical Research, 1993).

Test Agents. Unencapsulated DOX (Adriamycin 2.0 mg/ml; Farmitalia Carlo Erba, Milan, Italy) and CDDP (1.0 mg/ml; David Bull Laboratories, Victoria, Australia) were obtained from the Cytotoxic Drug Pharmacy, Hammersmith Hospitals National Health Service Trust. DTPA was obtained from Janssen Chimica, Geel, Belgium. All pegylated liposomal agents were supplied by SEQUUS Pharmaceuticals, Menlo Park, CA. PLED was provided with the following lipid composition (values expressed in % molar ratio): (a) hydrogenated soybean phosphatidylcholine (56.2%); (b) cholesterol (38.3%); and (c) 3 STEALTH liposomes are a registered trademark of the ALZA Corporation, Palo Alto, CA.
N-(carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (5.3%). The doxorubicin was contained in the internal aqueous core of the liposome in the presence of 250 mM ammonium sulfate at a drug:phospholipid ratio of \(125 \text{ mg/mg}\). In this preparation, the liposomes were suspended in a 10% sucrose solution with more than 95% of the drug encapsulated within the liposomes. The mean particle diameter as measured by dynamic laser light scattering was 96 nm (range, 80–110 nm). Supplies of PLED were stored at 4°C in the liquid phase at a drug concentration of 2 mg/ml. PLEC was supplied with a lipid composition as follows (values expressed in % molar ratio): (a) hydrogenated soybean phosphatidylcholine (51.0%); (b) cholesterol (44.0%); and (c) N-(carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (5.0%). The total lipid content was approximately 71 mg/ml.

This was attributable to the different drug-loading mechanisms for the two agents: passive encapsulation for PLEC and remote loading for PLED. However, it has been shown previously that the pharmacokinetics of pegylated liposomes are independent of lipid dose (19). Pegylated liposome encapsulated DTPA (Janssen Chimica, Geel, Belgium) was used as a form of “empty” liposome. This liposome had the same lipid formulation as that of the PLED liposome. Pegylated liposomal DTPA was supplied in sterile 20-ml vials at 22°C and was subsequently stored at this temperature until the time of use.

**Drug Administration.** For these studies, all test drugs were administered by i.v. bolus injection via the lateral tail vein on days 15–17 after tumor inoculation. DOX, PLED, CDDP, and PLEC were injected either as single doses of 2 mg/kg or as 3 doses of 0.67 mg/kg over 3 days to groups of mice \((n = 9–12)\). In the absence of supplies of liposomes with no encapsulated agent, pegylated liposomes (with the same lipid formulation as PLED) containing DTPA were used as a control. The aim was to assess the effect of the liposome vehicle (diluted to the same lipid dose as PLED) on the response of KB tumors to RT. Because these liposomes contained DTPA, additional controls were performed in which animals received unencapsulated DTPA (100 \(\mu\)l of 0.02% w/v).

### Table 1  Effect of single-fraction RT on KB xenograft tumors in nude mice. Median times to reach 3V₀ and statistical analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>4.5 Gy</th>
<th>9 Gy</th>
<th>13.5 Gy</th>
<th>18 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT alone</td>
<td>12.7</td>
<td>22.6</td>
<td>30.6</td>
<td>44.4</td>
</tr>
</tbody>
</table>

\(P^a\)

### Table 2  Effect of fractionated RT on KB xenograft tumors in nude mice. Median times to reach 3V₀ and statistical analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>9 Gy/3F</th>
<th>15 Gy/5F</th>
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</thead>
<tbody>
<tr>
<td>RT alone</td>
<td>13.2</td>
<td>18.9</td>
</tr>
</tbody>
</table>

\(P^b\)

### Table 3  Effect of single-dose DOX or PLED (2 mg/kg) plus single-fraction RT against KB xenograft tumors in nude mice. Median times to reach 3V₀ and statistical analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>4.5 Gy</th>
<th>9 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>12.7</td>
<td>22.6</td>
</tr>
<tr>
<td>DOX plus RT</td>
<td>15.2</td>
<td>30.5</td>
</tr>
<tr>
<td>PLED plus RT</td>
<td>24.6</td>
<td>35.4</td>
</tr>
</tbody>
</table>

\(P^c\)

\(a\) Median time taken to reach 3V₀ for untreated control was 7.3 days. N/A, not applicable.

\(b\) Wilcoxon rank-sum test, differences considered significant at \(P < 0.05\). N/A, not applicable.

\(c\) Median time taken to reach 3V₀ for untreated control was 7.3 days.
**Tumor Irradiation.** Tumor RT was performed using a 111 TBq \(^{137}\)Cs source (CIS Bio International, Gif-sur-Yvette, France) with the mice carefully positioned within a specially constructed jig, which is depicted in Fig. 1. Before animal RT studies were commenced, the system was calibrated with lithium fluoride TLDs (Nuclear Enterprises, Reading, United Kingdom), which themselves had been calibrated at known SFRT doses (3, 6, 9, 12, 15, and 20 Gy) on a 6-MV linear accelerator (Varian, Crawley, United Kingdom) in the Department of Clinical Oncology, Hammersmith Hospital, Hammersmith Hospitals National Health Service Trust. The absorbed radiation dose was determined by reading light output in a Toledo 654 TLD reader (D.A. Pitman, Weybridge, United Kingdom) to yield a standard curve (data not shown). Thereafter, TLDs from the same batch were used for the experiments.

Fig. 2 Response of KB xenograft tumors in nude mice to single-fraction doses of radiotherapy (4.5 Gy) in combination with either DOX or PLED (2 mg/kg). Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.

Fig. 3 Response of KB xenograft tumors in nude mice to single-fraction doses of radiotherapy (9 Gy) in combination with either DOX or PLED (2 mg/kg). Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.
were irradiated in the jig with dead tumor-bearing mice with TLDs attached to the skin over the site of the tumor acting as phantoms. The TLDs were placed at the estimated midplane of the tumor in the ventro-dorsal plane to give a mean tumor measurement. Using this set-up, the tumor was exposed to irradiation for a total of 10 min, and the absorbed radiation dose was determined as described above.

Before therapeutic RT, the animals were anesthetized with an i.p. injection of 100 μl of a 1:1:4 mixture of the neuroleptanalgesic Hypnorm (fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml; Janssen-Cilag, Ltd., High Wycombe, United Kingdom), the benzodiazepine sedative Hypnovel (midazolam 5 mg/ml; Roche Products, Ltd., Welwyn Garden City, United Kingdom), and water for injection BP (Fresenius Health Care Group, Basingstoke, United Kingdom). This well-established regimen has been shown to provide effective short-duration anesthesia and

![Fig. 4 Response of KB xenograft tumors in nude mice to fractionated doses of radiotherapy (9 Gy in 3 fractions) in combination with single doses of either DOX or PLED (2 mg/kg). Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.](image1)

![Fig. 5 Response of KB xenograft tumors in nude mice to fractionated doses of radiotherapy (9 Gy in 3 fractions) in combination with multiple doses of either DOX or PLED [0.67 mg/kg (x3)]. Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.](image2)
has the advantage of maintaining better tissue perfusion than barbiturate anesthesia (20). Furthermore, tumor blood flow is only slightly reduced with this combination of anesthetic agents (21). Anesthetized animals were positioned in the compartments of the irradiation jig with the s.c. xenograft tumors overlying the radiation aperture in the lead block, and the rest of the animal’s body was placed over the 4-cm-thick lead shielding. Considerable care was taken to avoid direct pressure on the tumor mass to minimize the risk of creating areas of pressure-induced hypoxia during RT, because this has been shown to influence the efficacy of this treatment. On average, mice were anesthetized for ~30 min. They were kept warm by means of a heat lamp after irradiation.

**SFRT and FRT.** The dose/response effect of SFRT was initially assessed by irradiating groups of mice with single-fractions of radiation at doses of 4.5 Gy (n = 17), 9 Gy (n = 12), 13.5 Gy (n = 8), and 18 Gy (n = 8) over a period of 6.4 to 25.7 min at a dose rate of 0.7 Gy/min, as determined by the dosimetric calibration detailed above. Similarly, groups of tumorbearing mice received daily FRT to a dose of either 9 Gy in three fractions over 3 days (9 Gy/3F; n = 11) or 15 Gy in five fractions over 5 days (15 Gy/5F; n = 10). Each fraction of RT was delivered over a period of 4.3 min at a dose rate of 0.7 Gy/min.

**SFRT plus Doxorubicin or Cisplatin.** Tumor-bearing mice received injections of either DOX, PLED, CDDP, or PLEC at a dose of 2 mg/kg 16 h before receiving a SFRT dose of either 4.5 Gy or 9 Gy.

**FRT plus Doxorubicin or Cisplatin.** The effect of combining FRT with DOX, PLED, CDDP, and PLEC was investigated according to two protocols. In the first design, tumor-bearing mice received injections of one of these agents at a dose of 2 mg/kg 16 h before commencing a fractionated course of RT up to a dose of 9 Gy/3F in 3 consecutive days. In the second design, the mice received the same dose of the test agent in divided doses over 3 days (i.e., 0.67 mg/kg each day), with each injection administered 16 h before tumor irradiation up to a dose of 9 Gy/3F over 3 consecutive days.

**SFRT plus Liposomal Vehicle or DTPA.** Tumor-bearing mice received injections of either pegylated liposomal DTPA or unencapsulated DTPA in a volume of 100 μl 16 h before receiving a SFRT dose of either 4.5 Gy or 9 Gy.

**Toxicity Evaluation.** Animals were weighed once a week in the period between tumor implantation and the start of treatment. Thereafter, they were weighed three times a week for 2 weeks and then twice a week until the completion of the study. Local RT-induced cutaneous toxicity was assessed by inspection of the skin in the radiation field at the time of tumor measurement. No attempt was made to obtain serial blood samples to assess hematological or biochemical toxicity.

**RESULTS**

**RT Alone.** The effect of SFRT doses of 4.5 Gy, 9 Gy, 13.5 Gy, and 18 Gy on the time taken for KB tumors to reach 3Vo is presented in Table 1. These studies clearly demonstrated the efficacy of SFRT in this tumor model. The times taken to reach 3Vo were significantly greater for each of the RT doses as compared with the untreated control group. Furthermore, comparison between the different RT groups revealed a dose-response relationship, although at the higher radiation doses of 13.5 Gy and 18 Gy the difference did not reach the level of statistical significance. The effect of FRT to doses of 9 Gy/3F in 3 days and 15 Gy/5F in 5 days on the time taken for KB tumors to reach 3Vo is presented in Table 2. These studies confirmed the efficacy of FRT in this tumor model with the times taken to reach 3Vo significantly higher for FRT compared with untreated controls. For the studies of combined RT and chemotherapy, the 9 Gy/3F in 3 day dose was selected because of the increased ease of administration of 3, as opposed to 5, i.v. injections.

**RT and Doxorubicin.** The effect of SFRT at doses of either 4.5 Gy or 9 Gy in conjunction with either DOX or PLED on the growth of KB tumor xenographs is presented in Figs. 2 and 3. The median times taken to reach 3Vo and the results of the statistical tests are presented in Table 3. These data demonstrated that both DOX and PLED 2 mg/kg enhanced the effect of 4.5 Gy SFRT. This effect was particularly strong for PLED combined with 9 Gy RT alone, although the effect was of borderline significance for PLED plus 9 Gy RT. However, as can be seen from Fig. 3, 4 of 11 tumors treated with a combination of PLED 2 mg/kg and 9 Gy were locally controlled at 60 days, compared with 0 of 12 in the 9 Gy-alone group. The effect of FRT to a dose of 9 Gy in 3F in combination with DOX or PLED (in two different treatment schedules) on the growth of KB tumor xenographs is shown in Figs. 4 and 5. The median times taken to reach 3Vo and the results of the statistical tests are presented in Table 4. These data showed that

<table>
<thead>
<tr>
<th>Group</th>
<th>Median time to 3Vo (days)</th>
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<tbody>
<tr>
<td>RT vs. DOX 2 mg/kg plus RT</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RT vs. PLED 2 mg/kg plus RT</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RT vs. DOX 0.67 mg/kg (×3) plus RT</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RT vs. PLED 0.67 mg/kg (×3) plus RT</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DOX 2 mg/kg plus RT vs. PLED 2 mg/kg plus RT</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DOX 0.67 mg/kg (×3) plus RT vs. PLED 0.67 mg/kg (×3) plus RT</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>PLED 2 mg/kg plus RT vs. PLED 0.67 mg/kg (×3) plus RT</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>
both DOX and PLED significantly enhanced the effect of FRT. The effect was significantly greater for PLED as compared with DOX, irrespective of the schedule of drug administration ($P < 0.001$ for each comparison). In addition, when the two schedules of PLED were compared, there was no significant difference between a single 2 mg/kg dose and three divided doses of 0.67 mg/kg ($P > 0.1$).

**RT and Cisplatin.** The effect of SFRT at doses of either 4.5 Gy or 9 Gy in conjunction with either CDDP or PLEC on the growth of KB tumor xenografts is presented in Figs. 6 and 7.

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**Fig. 6** Response of KB xenograft tumors in nude mice to single-fraction doses of radiotherapy (4.5 Gy) in combination with either CDDP or PLEC (2 mg/kg). Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.

**Fig. 7** Response of KB xenograft tumors in nude mice to single-fraction doses of radiotherapy (9 Gy) in combination with either CDDP or PLEC (2 mg/kg). Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.
DISCUSSION

This study is the first to report the effect of CCRT using pegylated liposomal agents in an animal tumor model. Recently, based on these data and a previous study showing efficacy of pegylated liposomal agents in SCCHN xenograft tumors (22), Phase I/II clinical trials of CCRT using PLED in patients with SCCHN and non-small cell lung cancer have shown this approach to be associated with acceptable toxicity (23, 24). In addition, we have recently completed a Phase I/II study in patients with SCCHN treated with PLEC and RT4. The results reported here demonstrate that PLED and PLEC are capable of enhancing the effect of both SFRT and short-course FRT. In an attempt to allow a meaningful assessment of the interaction between the test agents and RT, drug doses were chosen which had been shown to have only modest effects on KB tumors in previous studies (22). Nonetheless, at the dose used in these studies, PLED was significantly more active than DOX in this model, and this fact should be considered when interpreting these data. There was no difference in the efficacy of PLEC and CDDP. For the studies involving SFRT, the administration of both PLED and PLEC was shown to increase significantly the effect of 4.5 Gy SFRT, with each pegylated liposomal agent showing greater activity than the unencapsulated drug. In contrast, the data for PLED and PLEC combined with 9 Gy SFRT showed no statistically significant enhancement of effect compared with 9 Gy RT alone; although the effect was of borderline significance for PLED plus 9 Gy. This finding may reflect the fact that a 9 Gy single fraction of RT was sufficiently effective in this model to obscure any additional impact of drug treatment. Despite these findings, it is noteworthy that the combined modality treatment achieved local control at 60 days in 36% and 30% for PLED plus 9 Gy and PLEC plus 9 Gy, respectively, compared with 0% for 9 Gy RT alone.

In addition, both PLED and PLEC were shown to enhance significantly the effect of FRT. This effect was particularly apparent for PLED as compared with DOX, regardless of whether a single or divided dose schedule was used. The equivalence of the two schedules of PLED administration has implications for the clinical applicability of pegylated liposomal

Toxicity. The treatment was well tolerated. There was no evidence of cutaneous toxicity in the animals treated with RT, with or without the test agents. The animals treated with 9 Gy SFRT experienced reversible weight loss of <10% of body weight, which was maximal at day 7 and recovered by day 17. There was no evidence that administration of any of the study agents increased this effect (data not shown). Similarly, the animals treated with RT experienced reversible weight loss that was slightly more severe (up to 12.6%) and maximal at day 10 after the first fraction of RT. Again, there was no evidence that weight loss was exacerbated by any of the test drugs (data not shown).


The median times taken to reach 3Vo and the results of the statistical tests are presented in Table 5. These studies demonstrated that the addition of PLEC 2 mg/kg enhanced the effect of 4.5 Gy SFRT (P < 0.001), although this effect was not seen when PLEC was given in addition to 9 Gy SFRT (P > 0.1). On the other hand, unencapsulated CDDP did not enhance the effect of SFRT at either 4.5 Gy or 9 Gy. In fact, CDDP plus 9 Gy yielded results which were significantly worse than 9 Gy RT alone. A direct comparison between the two combination strategies revealed that PLEC plus RT was significantly more effective than CDDP plus RT at both dose levels (P < 0.001 and P < 0.02, respectively), although these data must be viewed in the light of the relatively poor performance of CDDP plus 9 Gy. The effect of FRT to a dose of 9 Gy in three fractions in combination with either CDDP or PLEC on the time taken for KB tumor xenografts to reach 3Vo is shown in Figs. 8 and 9. The median times taken to reach 3Vo and the results of the statistical tests are presented in Table 6. These data showed that PLEC significantly enhanced the effect of FRT irrespective of the schedule of drug administration [P < 0.01 for 2 mg/kg, P < 0.05 for 0.67 mg/kg (×3)]. In contrast, neither CDDP schedule enhanced the effect of fractionated RT (P > 0.1 for both comparisons). Direct comparison between PLEC plus RT and CDDP plus RT showed that PLEC plus RT was superior when three divided doses were given (P < 0.05) but only of borderline significance when single doses were used (0.1 > P > 0.05).

**Table 5** Effect of single-dose CDDP or PLEC (2 mg/kg) plus single-fraction RT against KB xenograft tumors in nude mice. Median times to reach 3Vo and statistical analyses

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<th>Group</th>
<th>Median time to 3Vo (days)</th>
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<tr>
<td></td>
<td>4.5 Gy</td>
</tr>
<tr>
<td>No drug</td>
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<td>PLEC plus RT</td>
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<table>
<thead>
<tr>
<th>Group</th>
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<tr>
<td></td>
<td>4.5 Gy</td>
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<tr>
<td>RT vs. CDDP plus RT</td>
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<tr>
<td>RT vs. PLEC plus RT</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CDDP plus RT vs. PLEC plus RT</td>
<td>&lt;0.001</td>
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<sup>a</sup> RT (9 Gy) alone was superior to RT plus CDDP.
agents in combination with RT. At present, most CCRT strategies involve multiple injections of radiosensitizing agents throughout the course of RT, either as conventional full-dose treatment at three weekly intervals (25) or as low-dose daily infusions or boluses on the days that RT is given (26). The ability to achieve sustained intratumoral release of radiosensitizers during a course of FRT after intermittent administration of pegylated liposomal agents represents a potentially favorable therapeutic approach that would be considerably more convenient than daily injections.

As regards local RT-induced toxicity, the use of pegylated liposomal agents with RT raises concerns. PLED causes cutaneous toxicity (palmar-plantar erythrodysaesthesia) and mucosal ulceration at dose intensities above 12.5 mg/m² (27), suggesting that it might accentuate the toxicity of RT. Reassuringly, there was no evidence of exacerbation of cutaneous RT toxicity in these studies (although it must be borne in mind that the RT doses involved were relatively low). Of greater importance are the data from clinical studies which have shown little or no increase in local RT-induced toxicity during radical courses of RT (23, 24, 28). The fact that most clinical cutaneous toxicity with PLED is manifest in the hands and feet provides additional reassurance because these areas are rarely included in radiation treatment portals.

Fig. 8 Response of KB xenograft tumors in nude mice to fractionated doses of radiotherapy (9 Gy in 3 fractions) in combination with single doses of either CDDP or PLEC (2 mg/kg). Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.

Fig. 9 Response of KB xenograft tumors in nude mice to fractionated doses of radiotherapy (9 Gy in 3 fractions) in combination with multiple doses of either CDDP or PLEC [0.67 mg/kg (x3)]. Data are expressed in the form of a survival curve with survival defined as the time taken for tumors to reach 3Vo.
Liposomal Chemoradiotherapy in a Xenograft Model

Table 6 Effect of single- or multiple-dose CDDP or PLEC plus fractionated RT against KB xenograft tumors in nude mice. Median times to reach 3Vo and statistical analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>Median time to 3Vo (days)</th>
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<tr>
<td>9 Gy in 3F</td>
<td>13.2</td>
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<td>CDDP 2 mg/kg plus RT</td>
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<td>PLEC 2 mg/kg plus RT</td>
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</tr>
<tr>
<td>PLEC 0.67 mg/kg (×3) plus RT</td>
<td>20.6</td>
</tr>
</tbody>
</table>

Group | P
-- | --
RT vs. CDDP 2 mg/kg plus RT | >0.1
RT vs. PLEC 2 mg/kg plus RT | <0.01
RT vs. CDDP 0.67 mg/kg (×3) plus RT | >0.1
RT vs. PLEC 0.67 mg/kg (×3) plus RT | <0.05
CDDP 2 mg/kg plus RT vs. PLEC 2 mg/kg plus RT | 0.1 > P > 0.05
CDDP 0.67 mg/kg (×3) plus RT vs. PLEC 0.67 mg/kg (×3) plus RT | <0.05
PLEC 0.67 mg/kg plus RT vs. CDDP 0.67 mg/kg (×3) plus RT | >0.1
PLEC 2 mg/kg plus RT vs. CDDP 0.67 mg/kg (×3) plus RT | >0.1

Table 7 Effect of single-dose unencapsulated DTPA or pegylated liposomal DTPA plus single-fraction RT on KB xenograft tumors in nude mice. Median times to reach 3Vo and statistical analyses

<table>
<thead>
<tr>
<th>Group</th>
<th>Median time to 3Vo (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 Gy</td>
<td>12.7</td>
</tr>
<tr>
<td>9 Gy</td>
<td>22.6</td>
</tr>
<tr>
<td>RT alone</td>
<td>11.2</td>
</tr>
<tr>
<td>DTPA plus RT</td>
<td>20.7</td>
</tr>
<tr>
<td>Liposomal DTPA plus RT</td>
<td>18.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 Gy</td>
<td>10.4</td>
</tr>
<tr>
<td>9 Gy</td>
<td>18.9</td>
</tr>
</tbody>
</table>

RT vs. DTPA plus RT | >0.1
RT vs. liposomal DTPA plus RT | >0.1

Reviewing the data displayed in Figs. 2–9, it is not possible to draw any firm conclusion about the nature of the interaction (if any) between the liposomal drugs and the RT. The data would be compatible with an addition of the cytotoxic action of the drug to that of the RT, although a true radiosensitizing (supraadditive) effect cannot be excluded definitively. However, although this distinction is of theoretical interest, it carries relatively minor significance in the clinical situation because the critical issue dictating the success or failure of CCRT strategies is the therapeutic index (5). The potential advantage of using PLED or PLEC as part of CCRT lies in the fact that the liposomal vehicle provides a means of delivering the agents selectively to the tumor tissue. This offers the attractive prospect of having greater concentrations of the radiosensitizing drug in the tumor than in the adjacent normal tissues, thus increasing the therapeutic index. At the radiation doses used (in both SFRT and FRT) there was no evidence of increased local cutaneous radiation toxicity or systemic toxicity with any of the drug formulations. Therefore, these studies demonstrate that pegylated liposomes have significant potential for future development as vehicles for targeted drug delivery in CCRT strategies.

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Kevin J. Harrington, Gail Rowlinson-Busza, Konstantinos N. Syrigos, et al.


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