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ORIGINAL ARTICLE

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Pegylated liposome-encapsulated doxorubicin and cisplatin in the treatment of head and neck xenograft tumours

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Abstract *Purpose*: To evaluate the in vitro and in vivo activity of unencapsulated doxorubicin (DOX) and cisplatin (CDDP) and their pegylated liposome encapsulated counterparts (PLED and PLEC) in a subcutaneous model of human squamous cell cancer of the head and neck. *Methods*: In vitro cytotoxicity was determined by means of the sulphorhodamine B assay and in vivo activity was assessed in terms of tumour growth delay following single intravenous doses of the various agents. Treatment-related toxicity was evaluated by means of serial weight measurement. *Results*: The IC_{50} values for DOX (12.1-fold) and CDDP (21.5-fold) were lower than for their liposome-encapsulated counterparts. When the two unencapsulated agents were compared, the IC_{50} value for DOX was 16-fold lower than that for CDDP. In the in vivo studies, liposomes containing DTPA (PLEDTPA) exerted no effect on KB xenograft tumours when compared to untreated controls ($P > 0.1$). PLED was significantly more effective than DOX at doses of 2 mg/kg, 4 mg/kg and 8 mg/kg ($P < 0.001$ for all comparisons). At the 8 mg/kg dose, 7/13 animals treated with PLED were free of disease at 60 days, compared to 0/12 treated with DOX. PLEC displayed superior activity in comparison to CDDP at the 4 mg/kg dose level ($P < 0.001$), although at doses of 2 mg/kg and 10 mg/kg this comparison only reached borderline statistical significance ($0.1 > P > 0.05$). The highest dose level of 20 mg/kg was fatal to all animals in the CDDP group but well-tolerated by the animals in the PLEC group. On the basis of serial weight measurements, both PLED and PLEC were shown to be tolerated better than DOX and CDDP. *Conclusion*: Both PLED and PLEC were shown to exert significant activity against head and neck xenograft tumours, with PLED showing particular efficacy.

Key words Cisplatin · Doxorubicin · Head and neck cancer · Pegylated liposome

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Introduction

The precise role of cytotoxic chemotherapy in the treatment of squamous cell cancer of the head and neck (SCCHN) remains controversial. Despite the impressive response rates of single-agent or combination chemotherapy in patients with previously untreated SCCHN, chemotherapy does not represent a radical therapeutic modality and no patient will be cured by the use of this treatment alone. The most active single agents include cisplatin, 5-FU, methotrexate, bleomycin, vinca alkaloids and the taxanes [10, 21, 22, 30, 38, 41, 49]. The poor prognosis of patients with locally advanced SCCHN treated with surgery, radiotherapy or combinations of these treatments has given impetus to studies examining the integration of cytotoxic chemotherapy into treatment strategies for this disease. Indeed, recently published overview analyses have demonstrated a survival advantage of between 8% and 12% for combinations of radiotherapy and concomitant chemotherapy in patients with SCCHN [1, 30]. Therefore, the concomitant chemoradiotherapy (CCR) approach is now one of the most actively investigated areas in the treatment of SCCHN. As in other treatment sites, however, the use of CCR carries an increased risk of acute and late complications [17]. As a consequence, the search for more active cytotoxic agents with more

acceptable toxicity profiles may represent a potentially fruitful avenue for improving the outcome of patients with SCCHN.

A number of studies have shown that cytotoxic drugs encapsulated within both nonpegylated and pegylated liposomes are active against a range of murine tumours and human xenograft tumour systems [11, 50]. Doxorubicin, daunorubicin, epirubicin, mitoxantrone, vincristine and platinum derivatives have all been shown to be effective in liposomal formulations [4, 12, 13, 27, 28, 31, 33, 42, 43, 44, 45, 46, 47]. Cytotoxic agents entrapped within pegylated liposomes have been shown to have improved pharmacokinetics [14, 23], increased tumour localization [14, 18, 19, 20, 36, 44], enhanced therapeutic efficacy [2, 19, 27, 45, 46] and attenuated toxicity profiles [31, 43] when compared to the corresponding unencapsulated agent. In clinical studies, pegylated liposomal doxorubicin has been shown to have substantial activity against AIDS-related Kaposi's sarcoma [32, 40] and breast and ovarian cancers [29, 35].

In the context of CCR for SCCHN, the increased tumour localization of pegylated liposomal agents offers the prospect of selective drug deposition within tumour tissue relative to that in adjacent normal tissues. If this were achieved, it would have the potential to reduce the dose-limiting local toxicity of CCR. Therefore, in these studies we have examined the activity of two pegylated liposomal cytotoxic drugs against SCCHN xenograft tumours in nude mice in an attempt to identify agents for further development in CCR studies.

Materials and methods

Test agents

All pegylated liposome-encapsulated agents were provided by SEQUUS Pharmaceuticals (Menlo Park, Calif.). Pegylated liposome-encapsulated doxorubicin (PLED) was provided with the following lipid composition (values expressed in percent molar ratio): hydrogenated soybean phosphatidylcholine (HSPC) (56.2%), cholesterol (38.3%), and *N*-(carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE) (5.3%). The doxorubicin was contained in the internal aqueous core of the liposome in the presence of 250 m*M* ammonium sulphate at a drug/phospholipid ratio of approximately 150 μg/μmol. 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.

Pegylated liposome-encapsulated cisplatin (PLEC) was supplied with the following lipid composition (values expressed in percent molar ratio): HSPC (51.0%), cholesterol (44.0%), and MPEG-DSPE (5.0%). The total lipid content was approximately 71 mg/ml. The cisplatin was contained within the central aqueous core of the liposome at a drug/lipid ratio of approximately 14 μg/mg. In this formulation, drug encapsulation exceeded 90% and the mean particle diameter was approximately 110 nm. Supplies of PLEC were stored in the dark at 4 °C as a liquid at a drug concentration of 1 mg/ml. Pegylated liposome-encapsulated diethylenetriaminepentaacetic acid (DTPA; Janssen Chimica, Geel, Belgium) (PLEDTPA) was used as a form of "empty" liposome. This liposome had the same lipid formulation as that of the PLED liposome. PLEDTPA was supplied in sterile 20-ml vials at −20 °C and was subsequently stored at this temperature until use.

Unencapsulated doxorubicin (DOX; Adriamycin 2.0 mg/ml; Farmitalia Carlo Erba, Milan, Italy) and cisplatin (CDDP) (cisdiamminedichloroplatinum 1.0 mg/ml; David Bull Laboratories, Victoria, Australia) were obtained from the Cytotoxic Drug Pharmacy, Hammersmith Hospitals NHS Trust. DOX was stored at 4 °C for a maximum period of 3 months before use. CDDP was stored in the dark at room temperature (approximately 16 °C) for a maximum period of 3 months before use.

In vitro cytotoxicity assay

The in vitro cytotoxicity of doxorubicin (both DOX and PLED) and cisplatin (both CDDP and PLEC) was measured using the sulphorhodamine B (SRB) assay [37]. The human tumour KB cell line was used in these studies. This cell line was derived from a male patient with a poorly differentiated squamous cell cancer of the floor of the mouth and tongue and established in cell culture in 1954 [9]. KB tumour cells were grown to subconfluence in 175-cm^2 tissue culture flasks (Falcon, N.J.) in RPMI-1640 medium containing penicillin 100 U/ml and streptomycin 100 μg/ml, supplemented with 10% fetal calf serum (FCS; Gibco, Paisley, UK) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Tumour cells were harvested by brief incubation with a 1:3 solution of trypsin/versene (EDTA 0.02%) and resuspended in 25 ml 2% RPMI medium. Culture medium, EDTA and trypsin were supplied by the Media Production Unit at the Imperial Cancer Research Fund, Clare Hall, UK.

Cell suspension (1 ml) was added to 2% RPMI (9 ml) supplemented with 10% FCS containing various concentrations of DOX (1.0 n*M* to 10.0 μ*M*), CDDP (1.0 n*M* to 100 μ*M*), PLED (1.0 n*M* to 500 μ*M*) or PLEC (1.0 n*M* to 1.0 m*M*) and incubated at 37 °C in an atmosphere containing 5% CO_2 for 24 h. Cells were then harvested and resuspended in 2 ml medium and 100 μl of the cell suspension was added to the first well of a 96-well plate, each well of which contained 100 μl medium. Serial one in two dilutions were performed down the plate. The plate was left to incubate for 96 h at 37 °C, after which the cells were fixed with trichloroacetic acid (TCA) to prevent their being dislodged by washing with buffer. Cells attached to the plastic substratum of the plate were fixed by gently adding 25 μl cold (4 °C) 50% TCA on top of the growth medium in each well to produce a final TCA concentration of 10%. Thereafter, the cultures were incubated for 1 h at 4 °C and then washed five times with tap-water to remove TCA, growth medium, serum protein and low molecular weight metabolites. TCA-fixed cells were stained for 30 min with 50 μl 0.4% (wt/vol) SRB dissolved in 1% acetic acid. After 30 min, the SRB was removed and the plates were rapidly rinsed four times with 1% acetic acid to remove unbound dye. After rinsing, the plates were air-dried until no standing moisture was seen. The protein-bound dye was then solubilized with 10 m*M* unbuffered Tris base (pH 10.5) for 5 min on a gyratory shaker. The optical density was read at 492 nm on a Titertek Multiskan MCC/340 plate reader.

KB tumour model

Female nude mice of mixed genetic background were used in these experiments. Animal husbandry procedures and establishment of tumour xenografts have been described previously [16]. Starting 7 days after tumour inoculation the tumours were measured on at least three occasions before the start of treatment. Three orthogonal tumour diameters corresponding to length, breadth and height (d_1, d_2 and d_3) were recorded using vernier calipers and the tumour diameter was calculated from the formula: $V = \pi/6 \times d_1 \times d_2 \times d_3$.

The tumour volume on the day of injection of the therapeutic agent was designated as the initial volume or V_0. Tumour volume was assessed two or three times per week on the following days (relative to the day of drug administration): −7, −4, 0, 3, 5, 7, 9, 11, 14, 17, 21, 24, 28, 32, 36, 40, 44, 48, 52, 56 and 60. The absolute and

relative (as compared to V_0) tumour volumes were calculated. Mice were killed after the tumour had increased in volume to more than three times its original volume ($3V_0$). The time taken to reach $3V_0$ was estimated by interpolation between the two data points immediately before and immediately after attainment of $3V_0$. This value was used as a surrogate measure of animal survival on the assumption that those tumours that had tripled their original volume were destined to increase in size inexorably. The use of this measure was designed to spare the animals from the physical distress of unnecessarily large tumour burdens and to comply with the Medical Research Council guidelines ('Responsibility in the Use of Animals for Medical Research', 1993).

Drug administration

All test drugs were administered as single doses to KB tumour-bearing mice by intravenous bolus injection via the lateral tail vein. The administered dose was calculated on the basis that the average weight of the nude mice used in these studies was approximately 25 g. Therefore, doses were administered on the basis that 1 mg/kg was equivalent to 25 μg of drug. PLED was administered to groups of mice at doses of 2 mg/kg ($n = 11$), 4 mg/kg ($n = 12$), and 8 mg/kg ($n = 13$). Similarly, DOX was administered to groups of mice at doses of 2 mg/kg ($n = 11$), 4 mg/kg ($n = 11$), and 8 mg/kg ($n = 12$). The volume injected varied from 100 μl to 200 μl. PLEC was administered to groups of mice at doses of 2 mg/kg ($n = 11$), 4 mg/kg ($n = 12$), 10 mg/kg ($n = 12$) and 20 mg/kg ($n = 12$). Similarly, CDDP was administered to groups of mice at doses of 2 mg/kg ($n = 10$), 4 mg/kg ($n = 12$), 10 mg/kg ($n = 12$) and 20 mg/kg ($n = 7$). The volume injected varied from 100 μl to 500 μl. Untreated control animals ($n = 25$) received no active therapy but, in other ways, were treated in an identical fashion to the animals in the treated groups. The animals treated with PLEDTPA ($n = 17$) received a 100-μl intravenous bolus injection.

Toxicity evaluation

Animals were weighed once a week in the period between tumour implantation and injection of the test agents. Thereafter, they were weighed three times a week for 2 weeks and then twice a week until the completion of the study. No attempt was made to obtain serial blood samples to assess haematological or biochemical toxicity.

Statistical analysis

Relative tumour volume was recorded for 15–25 days for each animal in a treatment group. The times taken to reach $3V_0$ were recorded as an indication of the progression of the xenograft tumours as described above. The Wilcoxon rank sum test was used to test the difference between the times taken to reach $3V_0$ in the various test groups. Differences were considered to be significant at $P < 0.05$.

Results

In vitro cytotoxicity assay

The results of the in vitro cytotoxicity assays are shown in Fig. 1a,b. The IC_{50} values were determined by fitting the data to a logistic sigmoid plot using the SigmaPlot 5.0 program (SPSS Science, Chicago, Ill.). The values for DOX and PLED were 0.0156 μM ($r^2 = 0.991$) and 0.189 μM ($r^2 = 0.993$), respectively. The corresponding values for CDDP and PLEC were 0.249 μM ($r^2 = 0.992$) and 5.35 μM ($r^2 = 0.998$), respectively. Both DOX (12.1-fold) and CDDP (21.5-fold) were more cytotoxic than their liposome-encapsulated counterparts. Interestingly, the IC_{50} value for DOX was found to be more 16-fold lower than that for CDDP.

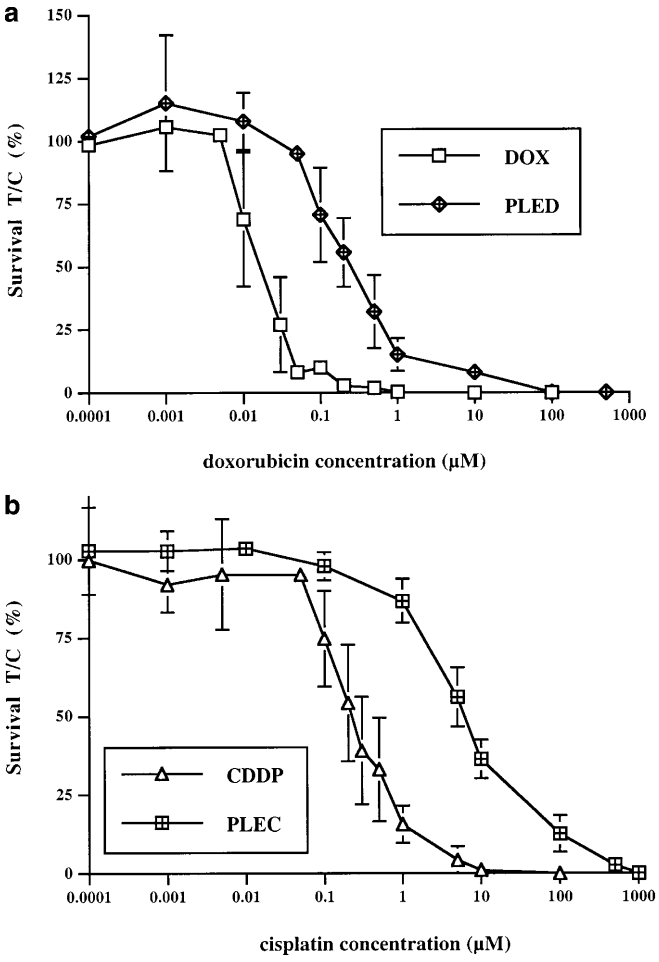

Fig. 1a,b In vitro SRB cytotoxicity assay for test agents against KB cells (**a**) DOX and PLED, and (**b**) CDDP and PLEC

Untreated controls and PLEDTPA

The times taken for the tumours in the two groups of animals to reach $3V_0$ are shown in Fig. 2. The median times to reach $3V_0$ for the untreated and PLEDTPA-treated groups were 7.3 and 8.6 days, respectively ($P > 0.10$). This confirmed that the liposomal vehicle exerted no intrinsic activity against KB SCCHN xenograft tumours.

DOX and PLED

The times taken for the tumours in the various groups treated with either DOX or PLED (2 mg/kg, 4 mg/kg and 8 mg/kg) to reach $3V_0$ are displayed in Fig. 3. The median times to reach $3V_0$ and the results of the statistical comparisons of the various treatment groups are

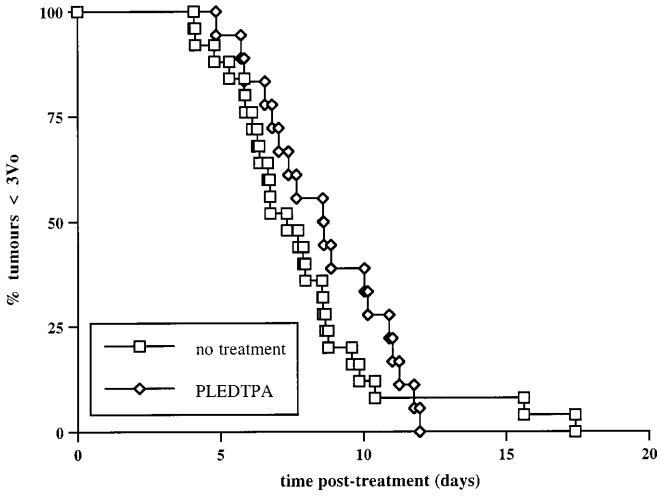

Fig. 2 Effect of PLEDTPA and no treatment on the time taken to reach $3V_0$ in KB xenograft tumour bearing nude mice

presented in Table 1. It can be seen that DOX was significantly more active than no treatment at the 2 mg/kg and 8 mg/kg dose levels, but not at the 4 mg/kg dose level ($P > 0.1$). PLED was significantly more effective than no treatment at all dose levels ($P < 0.001$ for all comparisons). Indeed, at the 8 mg/kg dose level, 7 of 13 KB xenograft tumours were locally controlled at 60 days. In all cases there was either complete resolution of the tumour with no regrowth or regression to a small nidus of residual tissue which showed no sign of recurrence. In addition, direct comparison of DOX and PLED at all three dose levels revealed that the liposomal agent was significantly more effective than the unencapsulated drug ($P < 0.001$ for all comparisons).

CDDP and PLEC

The times taken for the tumours in the various groups treated with either CDDP or PLEC (2 mg/kg, 4 mg/kg, 10 mg/kg and 20 mg/kg) to reach $3V_0$ are displayed in Fig. 4. No data were available for CDDP at a dose of 20 mg/kg beyond the 3-day time-point because of the death of all the animals in this group at that time. Post-mortem examination of this group of animals revealed cachexia with abdominal swelling which was found to be due to gross gastric distension by retained food. It would appear that these animals died of gastric outflow obstruction, presumably secondary to CDDP treatment. It can be seen that both CDDP and PLEC were active against KB xenograft tumours, especially at the higher doses. The median times to reach $3V_0$ and the results of the statistical comparisons of the various treatment groups are presented in Table 2.

These comparisons demonstrate the efficacy of both CDDP and PLEC when compared to untreated controls. However, the direct comparison between CDDP and PLEC shows that at only one dose level (4 mg/kg) was the difference between the agents statistically significant (although at the 2 mg/kg and 10 mg/kg dose

Fig. 3 Effect of DOX and PLED on the time taken to reach $3V_0$ in KB xenograft tumour-bearing nude mice

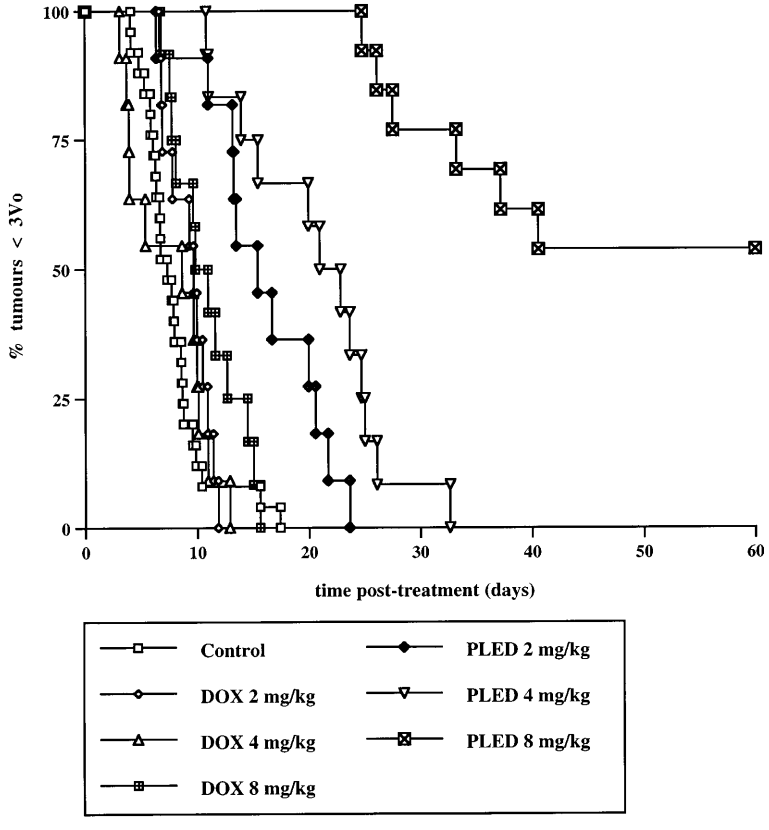

Table 1 Effect of single-dose DOX and PLED on KB xenograft tumours in nude mice

	Group						
	Control	2 mg/kg		4 mg/kg		8 mg/kg	
		DOX	PLED	DOX	PLED	DOX	PLED
Time to $3V_0$ (days)							
Median	7.3	9.7	15.4	8.7	21.9	10.4	60.0
Range	4.1–17.4	6.6–11.9	6.4–23.6	3.1–12.9	10.8–32.7	6.8–15.6	24.8–60.0[a]
P-values[b]							
Control vs DOX		< 0.05		> 0.1		< 0.01	
Control vs PLED			< 0.001		< 0.001		< 0.001
DOX vs PLED			< 0.001		< 0.001		< 0.001

[a] Of 13 tumours, 7 were locally controlled at 60 days
[b] Wilcoxon rank sum test; differences considered significant at $P < 0.05$

levels the difference was of borderline significance). Such a comparison was not possible at the highest dose level because of the toxic deaths of the animals which had received CDDP. However, at the 20 mg/kg dose only in 1 of 12 mice treated with PLEC was the tumour controlled. These data compare unfavourably with tumour control in 7 of 13 mice treated at the highest dose of PLED (8 mg/kg).

Toxicity evaluation

The data relating to weight change during the course of the various studies are presented in Figs. 5 and 6. From Fig. 5 it can be seen that at the early time-point of 5 days, the control group of mice had gained 0.8% weight as compared to baseline at day 0. The animals treated with DOX 4 mg/kg and DOX 8 mg/kg had lost weight compared with their baseline weight (−4.4% and −6.7%, respectively). Of the animals treated with PLED, those treated at a dose of 4 mg/kg had lost 1.2% of their baseline weight, whereas those treated with 2 mg/kg or 8 mg/kg had gained a similar amount of weight to the controls (1.6% and 2.4%, respectively). At the later time-point of 17 days, the control group had gained 8.4% weight compared to baseline. Animals treated with PLED 2 mg/kg, 4 mg/kg and 8 mg/kg had all gained weight (7.3%, 5.1% and 6.9%, respectively) as had those

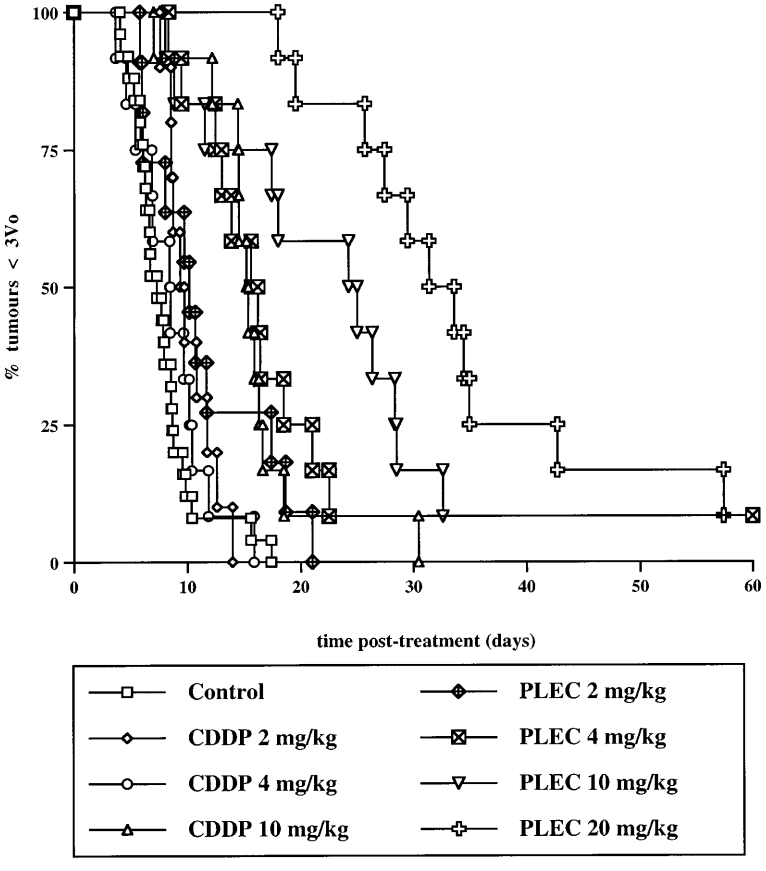

Fig. 4 Effect of CDDP and PLEC on the time taken to reach $3V_0$ in KB xenograft tumour-bearing nude mice

Table 2 Effect of single dose CDDP and PLEC on KB xenograft tumours in nude mice (*NA* data not available due to death of all animals in the CDDP 20 mg/kg group)

	Group								
	Control	2 mg/kg		4 mg/kg		10 mg/kg		20 mg/kg	
		CDDP	PLEC	CDDP	PLEC	CDDP	PLEC	CDDP	PLEC
Time to $3V_0$ (days)									
Median	7.3	9.6	10.2	8.5	15.9	15.3	24.7	NA	32.5
Range	4.1–17.4	7.6–14.0	5.8–21.1	3.7–15.9	8.4–60.0	7.1–30.5	8.1–60.0		18.0–60.0
P-values[a]									
Control vs CDDP		<0.01		>0.1		<0.001		NA	
Control vs PLEC			<0.05		<0.001		<0.001		<0.001
CDDP vs PLEC			$0.1 > P > 0.05$		<0.001		$0.1 > P > 0.05$		NA

[a] Wilcoxon rank sum test; differences considered significant at $P < 0.05$

mice treated with DOX 2 mg/kg and 4 mg/kg (9.6% and 1.6%). However, the weight of those mice treated with DOX 8 mg/kg had not recovered to baseline by this time (−2.0%).

From Fig. 6 it can be seen that animals treated with CDDP at doses of 10 mg/kg and 20 mg/kg suffered severe early weight loss (−11.1% at 5 days and −13.7% at 3 days, respectively). Indeed, none of the mice treated with CDDP 20 mg/kg survived beyond the 3-day time-point. In contrast, the 20 mg/kg dose of PLEC was associated with only minor weight loss (−0.8%) at 5 days. At the later time-point of 17 days, the animals treated with PLEC 2 mg/kg, 4 mg/kg, 10 mg/kg, and 20 mg/kg had all gained weight (7.5%, 6.9%, 3.5% and 5.1%, respectively) as had those mice treated with CDDP at 2 mg/kg and 4 mg/kg (8.4% and 6.6%, respectively). However, the mice treated with CDDP 10 mg/kg had not returned to baseline weight by this time (−0.8%).

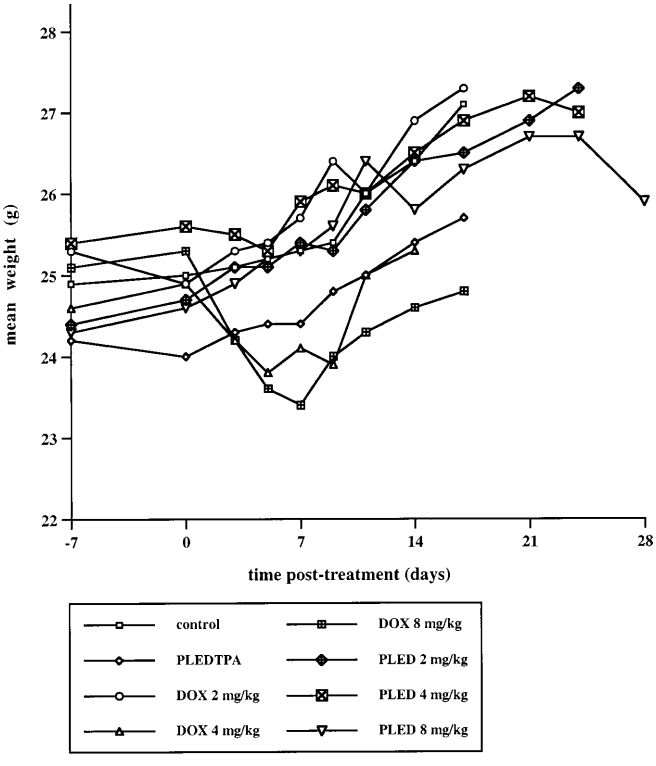

Fig. 5 Effect of treatment with DOX and PLED on the weight of KB tumour-bearing nude mice

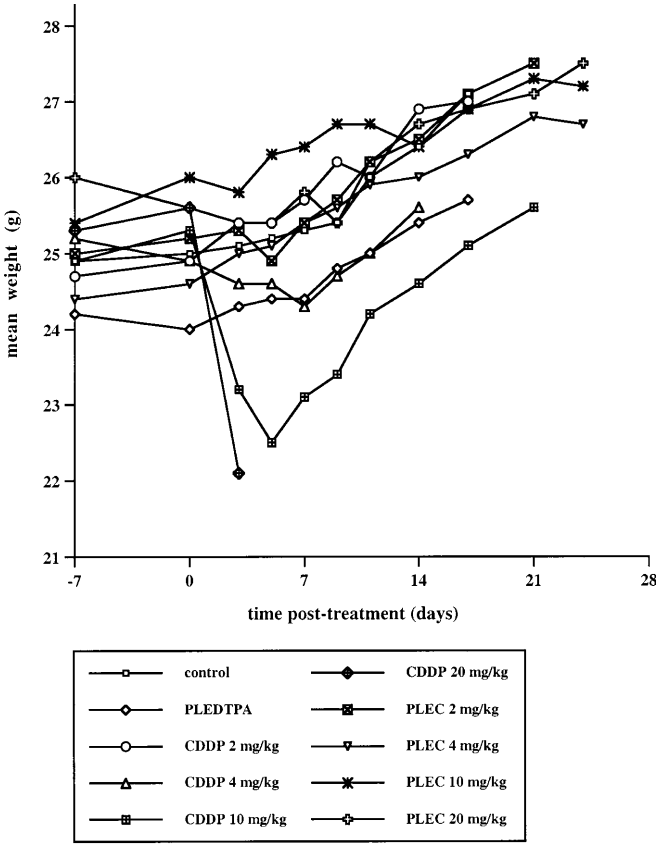

Fig. 6 Effect of treatment with CDDP and PLEC on the weight of KB tumour-bearing nude mice

Discussion

In these studies, DOX and CDDP were shown to be active against KB SCCHN cells in vitro. The finding

that DOX was more cytotoxic than CDDP (IC_{50} 0.0156 μM vs 0.249 μM) was rather unexpected, given the fact that anthracyclines are not considered to be amongst the most active group of drugs against SCCHN. In addition, the in vitro cytotoxicity data for PLED and PLEC followed this pattern with a difference in cytotoxicity of approximately 12-fold for DOX and PLED and approximately 20-fold for CDDP and PLEC. These data suggest that the in vitro cytotoxicity of each of these liposomal preparations is largely due to the presence of a low concentration of unencapsulated drug (approximately 5% free DOX in PLED and 10% free CDDP in PLEC). This, however, does not entirely explain the fact that the discrepancy between the IC_{50} values of the unencapsulated agents and of the liposomal forms was greater for PLEC than PLED since it might be expected that the presence of a greater percentage of unencapsulated drug in PLEC might have led to a relatively smaller difference than for PLED. Therefore, it is likely that there was some drug leakage over the 24-h period of in vitro exposure, with relatively greater leakage from the PLED liposomes than from the PLEC liposomes.

The data derived from the in vivo studies did not follow the same pattern as for the in vitro studies. The lack of antitumour activity of PLEDTPA confirmed that the pegylated liposomal vehicle did not possess any intrinsic cytotoxicity. DOX was found to possess only very modest activity against the SCCHN xenograft tumours, supporting the clinical impression that the anthracyclines are not a particularly active group of drugs in this tumour type. In contrast, PLED was shown to be the most effective agent against this tumour model and significantly more active than DOX at all three dose levels tested. By way of comparison, after a single dose of DOX 8 mg/kg all 12 tumours had reached $3V_0$ by 15.6 days, whereas after the same dose of PLED 7 of 13 tumours were effectively "cured". The explanation for this apparent discrepancy between the in vitro and in vivo data may lie in the fact that the clearance of DOX in vivo may be so rapid that the tumours are only minimally exposed to the drug, whereas in vitro continuous exposure over a period of 24 h is guaranteed. This would also explain the very significant in vivo activity of PLED since delivery of the drug in this form would lead to significant prolongation of the circulation half-life of the agent and increased intratumoral levels.

A review of the published literature reveals that doxorubicin and other anthracyclines have been used in the treatment of SCCHN. However, most of the studies have involved patients with locally advanced, recurrent or metastatic disease treated with combination regimens which include doxorubicin along with other active agents such as cisplatin and cyclophosphamide. This makes an objective assessment of the contribution of the anthracycline to the treatment effect rather difficult [5, 6, 7, 8, 34, 48]. There are no available data regarding the activity of single-agent doxorubicin either in previously untreated or relapsed disease. Martoni et al. [26] and Magee et al. [25] treated a total of 33 patients with locally advanced, recurrent and metastatic disease with single-agent epirubicin with a response rate of 12%, suggesting that anthracyclines possess activity against this group of tumours. Such data, taken in concert with those derived from these studies of SCCHN xenograft tumours in nude mice, suggest that further studies to examine the activity of this class of drug in patients with SCCHN are warranted.

The relatively poor showing of PLEC in this study was an unexpected finding, since CDDP is generally accepted as an essential element in all the currently used regimens for locally advanced, locally recurrent and metastatic SCCHN. The in vitro data demonstrated the sensitivity of this cell line to cisplatin and it might have been anticipated that the increased tumour localization of the drug achieved by liposome encapsulation would improve the treatment effect considerably, as was the case with PLED. However, although PLEC was shown to more active than CDDP, this finding only reached the level of borderline significance. The reason for this difference between the behaviour of PLED and PLEC requires explanation. One possible explanation may lie in the different behaviour of DOX and CDDP in vivo. DOX is cleared extremely rapidly from the circulation but CDDP is extensively protein-bound and has a longer circulation half-life [3]. Therefore, a relatively greater advantage may accrue from encapsulating DOX, as compared to CDDP, in pegylated liposomes.

Another possible factor is that the composition of the PLEC liposomes differed slightly from that of the PLED liposomes. This may have had two possible effects: (1) it may have adversely affected the tumour targeting properties of the PLEC liposomes; (2) it may have resulted in some alteration in the rate of drug release after localization of the liposomes within the tumour deposits. As far as the issue of drug release is concerned, the involvement of this factor seems to be supported by the findings of the in vitro studies which appeared to suggest that the PLEC liposomes were more stable than the PLED liposomes (as shown by the relatively greater in vitro cytotoxicity of PLED as compared to PLEC despite the greater amount of free drug in the PLEC formulation). Obviously, the in vitro situation does not provide an ideal model of the in vivo situation in that the medium used to sustain cell growth in vitro bears little resemblance to the in vivo milieu of the tumour interstitium. Nevertheless, the differential cell killing activity of the PLED and PLEC liposomes suggests that there may exist an intrinsic difference in the rate of degradation of these two preparations and this may, at least in part, explain the relative inactivity of PLEC.

Despite these findings that PLEC appears to confer a lesser therapeutic advantage than PLED when compared milligram for milligram with the unencapsulated drug, PLEC may be a useful agent. PLEC has been shown to have fewer systemic toxic effects than CDDP, as demonstrated by the difference in weight losses in the various treatment groups and the lack of treatment-

related mortality at the 20 mg/kg dose in these studies and the previously reported reduced nephrotoxicity and neurotoxicity in cynomolgus monkeys [51]. Therefore, this agent may be given at higher doses than those conventionally achieved with CDDP which may increase the levels of active drug delivered to tumours with resultant improved efficacy. Preliminary preclinical studies have also demonstrated the activity of PLEC in colonic and lung cancer models in mice [31, 47]. This potential to escalate the dose of PLEC is in direct contrast to the clinical experience with PLED, in which the occurrence of palmar-plantar erythrodysaesthesia as a novel dose-limiting toxicity has restricted dosing to a level (40 mg/m^2 every 3 weeks) which is lower than that achievable with DOX.

Whilst the findings of these studies are of interest, it is important to remember that the results of studies in a single cell line that has been propagated for many years in the laboratory and may have acquired a number of characteristics unrepresentative of this group of tumours may not be truly representative of head and neck cancers as a whole. The data obtained in this study certainly support the further evaluation of pegylated liposome targeted cytotoxic drug treatment strategies in SCCHN. As the meta-analyses [1, 30] have demonstrated, the main emphasis of that research should be directed towards evaluation of CCR approaches. This is likely to offer the best chance of pegylated liposomal agents demonstrating an enhanced activity profile in that the prospect of achieving selective tumour localization of the drug by means of the liposomal vehicle relative to the surrounding normal tissues within the radiation field means that there may be a beneficial therapeutic ratio in favour of the combined treatment. We have recently demonstrated the potential of this approach in the KB tumour xenograft model using PLED and a liposomal preparation of iododeoxyuridine [15]. In clinical studies, there exists the concern that delivery of PLED during irradiation of the tissues of the head and neck may result in unacceptable local tissue toxicity. This matter has recently been addressed in two phase I studies in patients with SCCHN who were able to receive PLED before and/or during courses of radical fractionated radiotherapy with acceptable toxicity [24, 39]. Further active exploration of this therapeutic approach is currently in progress in both preclinical and clinical studies in our laboratory.

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