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A phase I clinical evaluation of liposome-entrapped doxorubicin (Lip-Dox) in patients with primary and metastatic hepatic malignancy

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Liposome-entrapped doxorubicin (Lip-Dox) was evaluated in two phase I clinical trials in patients with hepatic malignancy. Patients with metastases from primary gastric or colonic tumours and patients with hepatoma were eligible. Lip-Dox was extremely well tolerated and acute toxicities such as nausea and vomiting were totally eliminated; no antiemetics were used even at doses of 80 mg/m^2. Toxicities such as alopecia and myelosuppression were also ameliorated. There were tumor regressions and reductions in hepatomegaly in patients treated on both the weekly and 21-day studies. The maximum tolerated dose (MTD) in the weekly study was 22.5 mg/m^2/week and in the 21-day trial the MTD was 70 mg/m^2.

Key words: Liposomes, doxorubicin, hepatic metastases, hepatoma, reduced toxicity.

Introduction

Liposomes are small phospholipid vesicles that circulate freely in the blood following i.v. injection; sequestration of liposomes occurs in structures served by a sinusoidal circulation. These vesicles may be loaded with cytotoxic drugs, and offer an attractive method whereby anticancer drugs can be effectively targeted to organs rich in reticuloendothelial cells such as the liver and spleen.

Liposomal delivery may also result in a delayed or controlled release of drug as well as altering the tissue distribution of the agent.[1,2] Altered tissue distribution and pharmacokinetic parameters have previously been demonstrated in animal models[3] in which clearly defined reductions in the toxicity of anthracyclines such as daunorubicin and doxorubicin were reported.

Plasma clearance of liposome-entrapped doxorubicin (Lip-Dox) is reduced, resulting in a greater area under the plasma concentration–time curve and there is also a diminished apparent volume of distribution.[1] Thus, blood levels are substantially higher and are sustained for greater periods than those found after the administration of free doxorubicin.

It has recently been stressed[4] that immediately liposomes enter the bloodstream they adsorb a coating of protein from the serum. Albumin, α and β globulins, clotting factors, high and low density lipoproteins, fibronectin, complement components and immunoglobulin G (IgG) have all been suggested as potentially adsorbed proteins. Some of these, most notably fibronectin and C-reactive protein, will increase the opsonization of the liposomes since macrophages have specific cell-surface binding sites for these molecules. Negatively charged liposomes are cleared from the bloodstream more rapidly than are those with neutral or positive charges[5], and this increased uptake may be related to a greater ability of the negatively charged liposomes to adsorb proteins.

Experimental studies using liposome-entrapped anthracyclines have shown unequivocal reductions in cardiotoxicity and that significantly increased survival times can be achieved.[6,7] Liposomes, in common with other small particulate drug delivery systems currently being studied, can only leave the circulation at sites where the endothelium is open or fenestrated. Thus, in addition to surface charge, variations in the size of the liposome can be used

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to specifically increase or decrease the uptake in chosen organs.

To enhance lung uptake, for example, liposomes of at least 5 μm are used, while to ensure a maximal uptake by hepatocytes the optimum size is less than 100 nm. The addition of receptor-site specific chemicals, e.g. lactosylceramide, may be used to increase the uptake of liposomes by hepatocytes through exploitation of the galactose receptor sites on these cells.[8]

Following the uptake of lipsome-entrapped doxorubicin by Kupffer cells *in vitro* unaltered doxorubicin was subsequently recovered from the culture medium.[9] The assay methods for the study enabled detection of doxorubicin metabolites but only minimal amounts of doxorubicinone and 7-deoxy-doxorubicinone were detected. The structure of doxorubicin is thus preserved not only in the acidic environment of the lysosome of the macrophage, but also during its subsequent passage out of the cell.

Liposomal targeting of antitumor drugs to organs such as the liver, spleen, lungs and bone marrow represents a potentially valuable procedure for the chemotherapy of primary and metastatic malignancies, particularly since the presence of secondary disease in the liver indicates a significantly poor prognosis. It has previously been shown that Lip-Dox was extremely well tolerated at doses of up to 20 mg/m^2/week[10,11] or 60 mg/m^2 every 21 days.[12]

The present paper reports the clinical evaluation of Lip-Dox in patients with primary and metastatic hepatic malignancies treated on both weekly and 21-day bolus regimes with doses up to 25 mg/m^2/week and 80 mg/m^2 every 21 days.

Materials and methods

The procedures adopted throughout this study were formally approved by the Medical Ethics Committee of the Royal Liverpool Hospital and the clinical trials were conducted under a Doctors and Dentists Exemption granted by the Medicines Control Agency of the Department of Health, London. Witnessed informed consent was obtained from each patient undergoing Lip-Dox therapy.

Formulation

Lip-Dox liposomes were prepared under aseptic conditions by hydration of phospholipids in an aqueous solution of doxorubicin, followed by extrusion throughout 0.2 μm pore-size polycarbonate nucleation-track membranes (Nuclepore). The phospholipids used were obtained as solutions in chloroform from Avanti Polar Lipids and were mixed to give egg yolk phosphatidyl choline and phosphatidyl glycerol in a 7:3 molar ratio. After addition of cholesterol and α-tocopherol hemisuccinate (Sigma, UK) to give a final mole ratio of 28% and 1.4%, respectively, the organic solution was depyrogenated by filtration through glass fibre and regenerated cellulose filters, then dried by rotary evaporation followed by exposure to high vacuum (below 1 Torr) on a lyophilizer overnight.

After hydration for 2 h by shaking with an isotonic solution of doxorubicin, the liposomes were then subjected to several passes through a Nuclepore 0.2 μm filter followed by sterile filtration through 0.22 μm Millipak filters. During and after processing, liposomes were stored under nitrogen at 4°C. Purity of phospholipids was confirmed by thin layer chromatography, quantitation was carried out using the Bartlett assay for phosphorus, and the extent of degeneration was examined under UV analysis, thio-barbituric acid assay and gas–liquid chromatography. Using these tests, the level of oxidation and hydrolysis of phospholipids in batches of the final product never exceeded 1%. The percent incorporation of doxorubicin in the liposome was measured by fluorescence after Sephadex G-50 minicolumn separation from free drug, and was found to be not less than 90%. The concentration of lipid in the final preparation was around 35 mg/ml and the concentration of doxorubicin was about 3 mg/ml.

Size analysis of liposomes was carried out by examining electron micrographs of negatively stained samples of the preparations dried down on carbon-coated formvar grids. Of the liposomes observed, 70% were in the size range 0.1–0.25 μm.

Sterility tests on samples at all stages of preparation were conducted in the Department of Medical Microbiology, University of Liverpool and were all reported as negative for the presence of microorganisms. All batches of Lip-Dox used clinically passed rabbit pyrogenicity tests (British Pharmacopoeia standard) carried out by Safepharm Ltd, Derbyshire, UK.

Patients

Patients eligible for Lip-Dox chemotherapy were those with primary hepatocellular carcinoma or

with hepatic metastases from primary gastric or colorectal adenocarcinoma. Histological confirmation of the tumour type was required prior to participation in the study.

Inclusion criteria

Life expectancy of greater than 2 months.
Karnofski status of 50% or greater.
Serum bilirubin 40 μmol/l or less.
White cells greater than 4×10^9/l.
Platelets greater than 100×10^9/l.
Identifiable marker lesion(s) on computed tomography (CT) or ultrasound scans.

Exclusion criteria

Prior chemotherapy.
Radiotherapy with past 4 months.
Significant cardiac disease.
Pregnancy.
Breast feeding.

Consent

Witnessed informed consent was sought from all patients deemed eligible for inclusion in the study. In a small number of cases an eligible patient, once informed of the nature of the disease, chose not to participate.

Patient evaluation

Patients accepted onto the study were given pre-treatment assessments that included ultrasonography, CT, full blood count and biochemistry (including total bilirubin, alkaline phosphatase, amino alanine transferase, γ-glutamyl transferase, albumin, globulin, creatinine, urea, bicarbonate, sodium, potassium and glucose).

In addition, a full clotting screen, urinalysis, electrocardiogram and either carcino-embryonic antigen (CEA) or α-fetoprotein (AFP) assays as appropriate, were performed together with a full physical examination. During treatment, haematology and biochemistry parameters were checked each week and patients were assessed for hepatomegaly every 3 weeks. Repeat ultrasound and CT scans were performed at appropriate intervals. All data were transferred to the clinical trial database (Clinical Computing Ltd, London; Operating System RSX 11-M+) and printed summaries made available for each patient prior to every ward admission or outpatient clinic.

Sixteen patients were treated during the weekly study and six on the 21-day bolus Lip-Dox trial. Their details are shown in Table 1. A further 12 patients were referred for the study but were excluded, usually because they were frankly jaundiced or had Karnofski scores of less than 50%.

Lip-Dox administration

For the weekly dose programme Lip-Dox was administered at the rate of 3 mg/min into a fast flowing saline drip in a forearm vein. Venous blood samples for the pharmacokinetic studies were taken from an indwelling cannula in the other arm. For the 21-day bolus study, Lip-Dox was administered by Graseby syringe driver at a rate of 4.5 mg/min into a fast flowing saline drip.

Table 1. Referrals, primary tumor type, mean age and treatment cycles of Lip-Dox patients

Weekly study			
Total referrals	26	Male	12
Accepted	16	Mean age	67.9
		Range	36–76
Primary tumor		Group mean	68.7
gastric	10	Female	4
colorectal	5	Mean age	71.0
hepatoma	1	Range	64–78
Treatment cycles			
singe cycle or less	7	15(2[a]), 20(2), 22.5(2), 25 mg/m^2/week	
two cycles	4	20, 22.5(2), 25 mg/m^2/week	
three or more cycles	5	15, 20(3), 25 mg/m^2/week	
21-day bolus study			
Total referrals	8	Male	5
Accepted	6	Female	1
		Mean age	60.2
Primary tumor		Range	51–68
gastric	2		
colorectal	3		
hempatoma	1		
Treatment cycles			
single	2	80 mg/m^2	
three	3	60, 70, 80 mg/m^2	
five	1	70 mg/m^2	

[a] (2) = number of patients at stated dose; 15(2) = two at 15 mg/m^2/week.

Maximum tolerated dose (MTD), dose range and escalation criteria

The determination of the MTD of Lip-Dox was based on treatment of patients in groups of three with escalation of dose between groups but not in individual patients. Confluent buccal ulceration, myelosuppression to less than 2×10^9/l total white cells or less than 100×10^9/l platelets were considered to be dose-limiting toxicities. Weekly Lip-Dox therapy was commenced at 15 mg/m^2 with escalation to 20, 22.5 and 25 mg/m^2 in subsequent groups allowed in the absence of the defined toxicities. In the results, patients are identified by reference to the dose and the enrolment number within that group (15-1, 20-2, etc.). On the 21-day schedule, therapy was started at 60 mg/m^2 with escalation to 70 and 80 mg/m^2 in subsequent groups. During the dose escalation an additional three patients were treated at any dose in which one of the initial three patients experienced any of the defined toxicities. The initial toxic dose (ITD) was considered to be that dose at which two or more patients within the expanded group of six experienced the defined toxicities. Cycles of therapy were repeated after 2 weeks without treatment during the first study and, in the event of recoverable myelosuppression, Lip-Dox was suspended until normal blood counts were restored.

Throughout all Lip-Dox administrations the patients were carefully observed and blood pressure, pulse and temperature measured regularly for up to 12 h following therapy. Hydrocortisone and chlorpheniramine were immediately available during all Lip-Dox administrations.

Results

Three patients died prior to completing their first full cycle of four treatments. One died from gastro-oesophageal bleeding, one from overwhelming malignancy and one from multiple pulmonary emboli following a deep vein thrombosis of the lower limb.

Side-effects of Lip-Dox therapy

A number of treatment-associated side-effects were observed during the studies and they are summarized in Table 2. Total administered doses and survival times from first treatment are summarized in Table 3.

Table 2. Observed side effects and tumor responses with Lip-Dox therapy

	No.	Patient details
Side effects		
gastro-intestinal	0	
red urine	1	(25-2)
allergic reaction	3	(20-3, 80-1, 80-3)
pyrexia	7	(15-1, 15-2, 20-1, 20-2, 25-1, 80-1, 80-2)
myelsuppression	4	(25-1, 25-2, 80-1, 80-3)
alopecia	9	(20-2, 22-3, 22-4, 60-1, 70-1, 70-2, 80-1, 80-2, 80-3)
Tumor response		
regression	3	(15-2, 25-2, 70-2)
stable disease[a]	7	(20-1, 20-2, 20-5, 22-1, 25-1, 70-1, 80-2)
progression	6	(15-1, 20-3, 20-4, 20-6, 22-3, 22-4)

[a] Patients subsequently showed progressive disease.

Gastro-intestinal effects. There was a striking absence of any adverse gastro-intestinal side effects during this trial. Throughout the course of both studies, in which a total of 119 individual doses of Lip-Dox were administered, no prophylactic antiemetics were used and not a single instance of nausea or vomiting was reported by any of the patients. No instances of buccal ulceration nor of diarrhoea were observed, even at the highest doses used in the studies.

Pyrexias and allergic reactions. During the weekly treatment study five patients (15-1, 15-2, 20-1, 20-2 and 25-1) experienced transient pyrexias about 10 h after treatment. These events were closely monitored and the patients were noted to show temperature rises to 39.5°C with increases in pulse and blood pressure. Shivering and sweating were observed and paracetamol (1 g stat with 1 g 4–6 h later) was routinely prescribed in these instances, all of which proved to be self-limiting.

One female patient (20-3) experienced an immediate allergic reaction to her first dose of Lip-Dox, becoming very flushed with a rapidly rising pulse and a sharp fall in blood pressure. Lip-Dox therapy was halted and she was given 10 mg chlorpheniramine and 100 mg hydrocortisone by i.v. injection. Within 45 min she had recovered completely and her treatment was recommenced the next day under prophylactic antihistamine cover. She experienced no further

Table 3. Sex, age, primary tumor, total dose, survival time and haematology data of selected patients

Patient	Sex	Age (years)	Tumor (response)[a]	Total dose (mg/m^2)	Survival time[b]	White cell nadirs ($\times 10^9/l$)	Platelets nadirs ($\times 10^9/l$)
15-2	M	66	G (R)	180	18	9.9	322
20-1	M	59	G (S)	400	47	2.9	367
20-2	M	70	C (S)	240	22	5.7	290
20-5	M	64	G (S)	240	23	4.2	295
22-1	M	50	C (S)	180	53	4.8	367
25-1	F	64	G (S)	300	36	1.8[c]	130
25-2	M	54	H (R)	200	17	3.4	52[c]
70-1	M	54	G (S)	210	13	2.9	121
70-2	M	67	H (R)	350	48	1.9[d]	60[d]
80-1	F	64	G (—)	80	4	0.7[c]	70[c]
80-2	M	57	C (S)	240	16	2.8	127
80-3	M	51	C (—)	80	6	1.5[c]	375

[a] G = gastric, C = colorectal, H = hepatoma; (R) = regression, (S) = stable, (—) = single treatment only so not eligible for evaluation.
[b] Survival in weeks from first Lip-Dox administration.
[c] Protocol-defined myelosuppression (doses 1–3).
[d] Myelosuppressed after five doses.

adverse reaction and the same routine was adopted for each of her subsequent treatments.

One 64-year-old male patient (20-5) experienced a mild anxiety reaction during his initial treatment which was suspended and restarted 30 min later when he felt more comfortable. Subsequently, he was given 25 mg thioridazine orally prior to each treatment.

Two of the patients treated every 21 days (80-1 and 80-2) experienced pyrexias following their initial Lip-Dox treatments. Again, these events proved transient and self-limiting.

Two patients (80-1 and 80-3) experienced immediate allergic reactions to Lip-Dox therapy. Following established practice, treatment was halted and i.v. antihistamines were given. Treatment was restarted 24 h later under antihistamine cover and neither patient experienced further reactions.

Alopecia. Alopecia was noted as a mild consequence of 10 doses of Lip-Dox at 20 mg/m^2 over a period of 15 weeks and after 4 doses in each of two patients treated at 22.5 mg/m^2. Alopecia was more common in the patients treated every 21 days and was clearly dose-dependent. All three patients treated at 80 mg/m^2 experienced total hair loss whilst those treated at 60 and 70 mg m^2 experienced only mild alopecia after three or more treatments. In all but one instance (80-1), the patients were males, aged over 50 years and with some evidence of pre-treatment hairline recession.

Myelosuppression. Myelosuppression was noted in two of three patients at 25 mg/m^2/week. In patient 25-1 the white cell nadir was $1.8 \times 10^9/l$ and in patient 25-3 the white cell nadir was $3.4 \times 10^9/l$ but accompanied by a platelet nadir of $52 \times 10^9/l$.

After single doses of 80 mg/m^2 two patients experienced marked myelosuppression. Patient 80-1 had a white cell nadir of $0.7 \times 10^9/l$ with a platelet count of $70 \times 10^9/l$ and patient 80-3 experienced a white cell nadir of $1.5 \times 10^9/l$ without an accompanying thrombocytopenia. In addition, after five treatments at 70 mg/m^2, patient 70-2 showed a marked degree of thrombocytopenia ($60 \times 10^9/l$).

Determination of maximum tolerated dose

The study protocols stipulated that in the event of myelosuppression or of confluent buccal ulceration in two or more patients treated at any dose, that dose would be deemed toxic and the MTD would be defined as the next lower dose.

In the first study the MTD was set at 22.5 mg/m^2/week during escalation from 15 to 25 mg/m^2/week and in the second trial the MTD was set at 70 mg/m^2 following reduction from the defined toxic dose of 80 mg/m^2.

Evaluation of clinical efficacy

Although designated as phase I studies, the antitumor efficacy of Lip-Dox was evaluated in the

majority of patients. Index hepatic lesions were measured clinically by ultrasound or CT scans during treatment. In one patient (15-2) malignant hepatomegaly was reduced by 6 cm and in another (25-2) by 3 cm. In the latter case there was also CT scan evidence of tumor shrinkage. Stable disease (defined as less than 10% growth of marker lesions in the absence of new deposits) was noted in patients 20-1, 20-2, 20-5, 22-1 and 25-1. These periods of stable disease lasted for between 6 and 10 weeks but were followed by progressive disease in all instances.

During the treatment of patient 70-2, who presented with hepatoma, the complete disappearance of tumour deposits from the left lobe of his liver was noted after the second treatment. A large, centrally necrotic lesion in the right lobe neither shrank nor grew throughout five cycles of Lip-Dox.

Patients 70-1 and 80-2 were both deemed to have experienced stable disease status after one and two treatments respectively; both, however, later showed progressive disease.

Discussion

It is useful to compare the results obtained using Lip-Dox with the anticipated consequences[13] of administering free doxorubicin over the dose range of 60–80 mg/m^2. With the free drug, approximately 80% of patients would be expected to experience nausea and vomiting, with about 30% experiencing these side effects severely. None of our patients suffered any nausea or vomiting and antiemesis was unnecessary. In further contrast, the free drug would be expected to produce some degree of mucositis in 20% of the treated patients, with perhaps 10% experiencing a severe manifestation. With Lip-Dox, no instances of even mild mucositis were seen. Myelosuppressive toxicity can be expected in some 30% of all treated patients given free doxorubicin at doses between 60 and 75 mg/m^2; however, with Lip-Dox, myelosuppression was not observed until patients were treated at 80 mg/m^2. Bone marrow depression in these Lip-Dox study patients was of short duration, less than 5 days, and usually of a degree quite acceptable in oncological practice. Alopecia, however, occurs in Lip-Dox patients as it does in those treated with free doxorubicin. Dose-dependent hair loss was a common feature of both our studies, most markedly in the 21-day bolus dose trial.

Thus the liposomal formulation has been demonstrated to be extremely well tolerated in patients with advanced malignant disease with hepatic involvement. These results lend unequivocal support to the observations of the several groups who, using animal models in pre-clinical studies, have demonstrated significant reductions in toxicity through liposomal delivery.[3,6,7,14] Cardiotoxicity was not evaluated in the two reported studies as we did not reach the currently accepted cumulative cardiotoxic dose of 450–500 mg/m^2 but the defined MTD of 70 mg/m^2 fits well with the 60 mg/m^2 MTD of the Roswell Park study.[12] During clinical studies in Israel myelosuppression to less than 2×10^9 was noted at 70 mg/m^2 but, due to protocol differences, evaluation of higher doses was permitted and a final MTD of 100 mg/m^2 was reported.[15]

Evaluation of tumour responses on the basis of CT and ultrasound scan measurements of marker lesions or of malignant hepatomegaly produced interesting and significant data. In both of the hepatoma patients there was demonstrable tumour regression. Hepatoma is usually considered to be susceptible to doxorubicin therapy and therefore tumour responses to Lip-Dox therapy demonstrates the potential value of the formulation in the treatment of this disease. That such treatment can be given with virtually no side effects represents an important contribution to the therapy of hepatoma, particularly in patients with advanced disease for whom chemotherapy with the free drug may not be considered an acceptable option.

In addition to these responses there were a number of instances of stable disease status observed in patients with metastatic tumours treated with Lip-Dox. In one instance there was clear evidence of a significant reduction in hepatomegaly in a patient with primary gastric adenocarcinoma. Four other patients with gastric primary disease and three with primary colorectal tumors all went through periods of apparently arrested tumour growth.

From the data generated during these phase I studies there appear to be no significant advantages to be gained from adopting the weekly treatment schedule. However, a more rigorous assessment of the clinical efficacy of Lip-Dox at the respective MTDs might reveal differences between the two alternative therapy schedules.

Conclusion

These results, particularly in patients with colorectal disease, for which doxorubicin is not

normally considered the drug of choice, suggest that there may have been significantly higher drug uptake in the liver following liposomal delivery. Alternatively, the action of Lip-Dox on tumor types not normally considered to be susceptible to the free drug may relate to the postulated sustained-release mechanism of the liposomal agent.[1,16]

Under such circumstances the cell-cycle stage specific action of anticancer drugs would be enhanced, particularly for such drugs as doxorubicin which have very short plasma half-lives, by exposure of susceptible cells within the tumor mass to drug for extended periods of time. Sustained plasma levels following liposomal delivery of anthracyclines, perhaps as the result of altered tissue distribution, have previously been reported.[3,15]

Our conclusion that the therapeutic index of doxorubicin can be substantially improved by adopting the liposomal delivery technique justifies further investigation of the role of liposomal delivery of antitumor drugs. Research into liposomal formulations with greater specificity for liver targeting and reduced entry into the sinusoidal circulation of the bone marrow is one area to which attention needs to be devoted. Additional studies on the pharmacokinetics, supplemented by radiolabeling, would be of considerable value in determining the fullest scope of liposomal delivery of anticancer agents and in the selection of the most appropriate treatment schedules.

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References

1. Gabizon A. Liposomes as drug delivery systems in cancer chemotherapy. In: Roerdink FHD, Kroon AM, eds. *Drug Carrier Systems*. Chichester: John Wiley 1989; 185–211.
2. Price G, Aherne W, New RRC, *et al.* Encapsulation of cytosine arabinoside in liposomes; a method of manipulating *in vivo* pharmacokinetics in man. *Proc Am Soc Cancer Res* 1989; **30**: 988.
3. Mayhew EG, Papahadjopoulos D. Therapeutic applications of liposomes. In: Ostro MJ, ed. *Liposomes*. New York: Marcel Dekker 1983: 289–341.
4. Juliano RL. Liposomes as drug carrier systems in the therapy of infectious diseases. In: Roerdink FHD, Kroon AM, eds. *Drug Carrier Systems*. Chichester: John Wiley 1989; 249–79.
5. Bonte F, Hsu MJ, Papp A, *et al.* Interactions of polymerizable phosphatidylcholine vesicles with blood components; relevance to biocompatibility. *Biochem Biophys Acta* 1987; **900**: 1–9.
6. Gabizon A, Goren D, Fuks Z, *et al.* Enhancement of adriamycin delivery to liver metastatic cells with increased tumoricidal effect using liposomes as drug carriers. *Cancer Res* 1983; **43**: 4730–5.
7. Mayhew EG, Goldrosen MH, Vaage J, *et al.* Effect of liposome-entrapped doxorubicin on liver metastases of mouse colon carcinomas 26 and 38. *J Natl Cancer Inst* 1987; **78**: 707–13.
8. Scherphof GL, Spanjer HH, Derksen JTP, Lazar G, Roerdink FHD. Targeting of liposomes to liver cells. In: Roerdink FHD, Kroon AM, eds. *Drug Carrier Systems*. Chichester: John Wiley 1989; 281–91.
9. Storm G, Regts J, Beijnen JH, *et al.* Processing of doxorubicin-containing liposomes by liver macrophages *in vitro*. *J Liposome Res* 1989; **1**: 195–210.
10. Sells RA, Owen RR, Gilmore IT, *et al.* Reduction in toxicity of doxorubicin by liposomal entrapment. *Lancet* 1987; **II**: 383.
11. Sells RA, Gilmore IT, Owen RR, *et al.* Reduction in doxorubicin toxicity following liposomal delivery. *Cancer Treat Rev* 1987; **14**: 383–87.
12. Greaven PJ, Cowens JW, Ginsberg R, *et al.* Clinical studies with liposomal doxorubicin. *J Liposome Res* 1990; **1**: 481–6.
13. Saletan S. Mitoxantrone; an active new antitumour agent with improved therapeutic index. *Cancer Treat Rev* 1987; **14**: 297–304.
14. Gabizon A, Meshorer A, Barenholtz Y. Comparative long-term study of the toxicities of free and liposome-associated doxorubicin in mice after intravenous administration. *J Natl Cancer Inst* 1986; **77**: 459–69.
15. Gabizon A, Amselem S, Goren D, *et al.* Preclinical and clinical experience with a doxorubicin-liposome preparation. *J Liposome Res* 1990; **1**: 491–8.
16. Storm G, Roerdink FHD, Steerenberg PA, *et al.* Influence of lipid composition on the antitumour activity exerted by doxorubicin-containing liposomes in a rat solid tumour model. *Cancer Res* 1972; **47**: 3366–72.

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