Targeted Delivery of Doxorubicin via Sterically Stabilized Immunoliposomes: Pharmacokinetics and Biodistribution in Tumor-bearing Mice

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Purpose. To evaluate benefits in tumor localization, availability, and noncancerous organ distribution of doxorubicin (DOX) delivered via small (<120 nm) sterically stabilized immunoliposomes targeted against a tumor-associated antigen in fibrosarcoma-bearing mice.

Methods. DOX-loaded liposomes were prepared with (i) specific monoclonal IgG2 antibody (32/2, D-SSL-32/2); (ii) non-specific IgG (D-SSL-IgG); or (iii) no IgG (D-SSL) on their surface. Equal DOX amounts were injected intravenously via each type of liposome into BALB/c mice carrying experimental lung metastases of a polyoma virus-induced fibrosarcoma (A9 ctc 220) expressing a polyoma virus-induced tumor-associated antigen (PAA) on their surface. Metastases occurred mainly in lung. Mice were treated at 3 stages of tumor development (micrometastases, medium-size metastases, and large, necrotic metastases). Performance evaluation was based on time-dependent quantification of DOX and DOX metabolites (DOX-M) in lung tumor, noncancerous organs, and plasma.

Results. (i) DOX delivered via both SSL retained the prolonged circulation time typical of DOX delivered via D-SSL. (ii) DOX accumulation in noncancerous organs was similar for all preparations. Low levels of DOX-M were obtained for all three preparations in all organs except liver, suggesting a similar processing. (iii) Preparations differed in behavior in lung tumor depending on tumor size and microanatomy. Only at the micrometastases stage were the specifically targeted D-SSL-32/2 superior to D-SSL and D-SSL-IgG, delivering 2–4 times more drug into the tumor. (iv) DOX-M level in all three tumor stages was in the following order: D-SSL-32/2 >> D-SSL >> D-SSL-IgG, suggesting that DOX delivered as D-SSL-32/2 is most available to tumor cells.

Conclusions. The advantage of specific targeting of sterically stabilized liposomes is expressed mainly in increasing availability of DOX to tumor cells in a way which is dependent on tumor microanatomy. The impact of this advantage to therapeutic efficacy remains to be determined.

KEY WORDS: sterically stabilized immunoliposomes; targeting; doxorubicin; lung metastases; pharmacokinetics; biodistribution.

INTRODUCTION

Doxorubicin (DOX)-loaded sterically stabilized liposomes (SSL) of ~100 nm deliver much higher levels of drug to tumors in animals (1) and humans (1,2) than are obtained with the free drug. However, accumulation of DOX-loaded SSL (D-SSL) in solid tumors is rather slow; for example, DOX peak levels in human tumors occur 3–6 days after D-SSL administration (2). D-SSL performance might be improved by active targeting, using a ligand on its surface which recognizes the tumor cells, thereby facilitating binding of the liposomes and subsequent drug uptake by the tumor cells. These liposomes are referred to as ligandoliposomes, and when the ligands are antibodies, as immunoliposomes. With systemically administered antibody-coated conventional liposomes, targeting to most solid tumors was not feasible, due mainly to the rapid uptake of the liposomes by the reticuloendothelial system (RES) (3,4). This obstacle to liposome targeting may be overcome by using small (<120 nm) SSL since their integrity in serum is maintained and RES uptake is dramatically reduced (1,5).

Sterically stabilized immunoliposomes (SSL) prepared in various ways (6–9), retaining both immunospecificity and steric stabilization, can be targeted to intravascular components (7,8). Moreover, in at least one tumor model in mice it was demonstrated that D-SSL are more efficacious than D-SSL in treating small metastatic solid tumors (10).

This study is aimed at evaluating three steps in comparative evaluation of D-SSL and D-SSL in tumor-bearing mice: (i) plasma, organ, and tumor pharmacokinetics of encapsulated DOX; (ii) drug availability to cells as assessed from rate and extent of DOX metabolism in noncancerous and cancerous tissues; and (iii) effect of tumor size and microanatomy on (ii).

MATERIALS AND METHODS

Reagents for Liposome Preparation

Sources, preparation and characterization of reagents, including lipids and antibodies, were as previously reported (9,11).

Cell Lines

A9 ctc 220 tumor cells (referred to as A9 cells) that were used as the specific target for binding IgG2 mouse MAb N132/2/4 (32/2) are similar to those described by Emanuel et al. (9) except that this tumor subline has a lower metastatic capacity and a lower density of PAA on its surface.
Animals

Specific pathogen-free BALB/c female mice, aged 9–12 weeks, were obtained from Harlan Sprague Dawley (Indianapolis, IN). Groups consisted of 4-6 mice for each time point.

Tumor Model

BALB/c mice were inoculated iv with 5 x 10^6 A9 cells to obtain experimental metastases. These cells settled and metastasized only in the lungs. Three different tumor stages as defined by size of lung metastases were studied: Stage I—micrometastases (tumor weight ≤ 0.01 g), Stage II—medium-size metastases (0.05–0.1 g), Stage III—large, necrotic metastases (≥ 0.8 g), occurring 14, 20, and 26 days post tumor inoculation, respectively. Without treatment, most mice died of lung tumors 35–45 days post inoculation.

Preparation of Sterically Stabilized Liposomes (SSL) and Immunoliposomes (SSIIL)

SSL and SSIIL loaded with DOX (D-SSL and D-SSIIL, respectively) or fluorescently labeled with FITC-PE were prepared and characterized as described elsewhere (9,11,12). In some experiments, conventional liposomes (Lip) were also used.

Pharmacokinetic and Biodistribution Studies in Tumor-Bearing Mice

The plasma pharmacokinetics included studies of the time-dependent clearance of: (i) liposomes without DOX, using 0.1 mole % FITC-PE-labeled SSL, SSL, and Lip; and (ii) DOX delivered as free drug and as D-SSL-32/2, D-SSIIL-IgG, D-SSL, and D-Lip. All liposomes and free DOX were diluted in sterile pyrogen-free 10% sucrose solution and injected (0.2 ml) into the tail veins of ~20-g mice. For the DOX-containing liposomes, each mouse received 35 μg DOX and ~0.35 μmoles phospholipids.

At the desired time, blood (0.5 ml) was collected from the retroorbital sinus after ether anesthesia into test tubes containing K3-EDTA as anticoagulant, and plasma was separated by centrifugation. A similar hematocrit of ~50% was obtained for all blood samples. Mice were immediately sacrificed, and their organs removed, washed in cold phosphate-buffered saline, dried over filter paper, and weighed. Plasma and organs were either processed immediately as described below or kept frozen at −70°C until analysis.

Aliquots of plasma of mice injected with FITC-PE-labeled liposomes (SSIIL-32/2, SSIIL-IgG, and SSL) were diluted in alkaline (borate buffer) isopropanol pH 9.5 to the range in which fluorescence (excitation 495 nm, emission 525 nm) is proportional to FITC-PE concentration. A calibration curve in saline/isopropanol pH 9.5 was used to determine FITC-PE concentration in plasma.

Extraction of DOX and DOX metabolites (DOX-M) from plasma and organs was performed as described by Cummings and McArdle (13). DOX and DOX-M were analyzed by HPLC (2.9). Daunorubicin (retention time [RT], 8.00 min) was used as internal standard. Synthetically prepared doxorubicin (DOL) (RT, 3.16 min), 7-deoxydoxorubicin glycol (7-deoxydoxorubicin-one, 7d-DOLON) (RT, 1.95 min), and 7-deoxydoxorubicinone (7d-DOXON) (RT, 2.83 min) were used as markers.

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RT of DOX was 4.46 min (2). Recoveries were always >95% and could be corrected by using the internal standard. HPLC assay sensitivity limit was 0.5 ng per peak (equivalent to 6.25 ng/ml plasma (~0.018% of injected dose)).

The identification of DOX-M was confirmed by TLC analysis (2). Blood volume for mice was assumed to be 77.8 ml/kg body weight; tissue DOX and DOX-M concentrations were corrected for plasma volume (14).

Nonlinear least-squares analysis was performed on pharmacokinetic and biodistribution data using Rstrip software (Micromath, Salt Lake City) (2).

Statistical Evaluation

The significance of the data was evaluated using Student’s t-test (two-tailed). P values < 0.05 were considered significant.

RESULTS

Plasma Pharmacokinetics

The two D-SSL preparations used in these experiments behaved similarly in tumor-bearing mice irrespective of antibody specificity, but differed from the D-SSL to a larger extent than in normal mice with respect to DOX plasma pharmacokinetics (data for Stage II are shown in Fig. 1 and Table 1). While clearance of DOX delivered via D-SSL was nearly monophasic, it was clearly biphasic for DOX delivered via D-SSIIL. Despite the initial fast clearance, >10% of the drug delivered via D-SSIIL remained in the plasma 24 h post-injection.

Total DOX-M level in plasma was very low for all three lipidosome preparations (Fig. 1); however, the lowest level was obtained with D-SSL, plateauing at 1% of the injected dose (ID) 6 h post-injection, compared with 2–2.5% for the two D-SSIIL preparations (p < 0.05).

To follow the plasma pharmacokinetics of the carrier itself without the drug, Lip, SSL, and SSIIL were labeled with 0.1 mole % nontransferable liposome marker FITC-PE (9,11). The pharmacokinetics of FITC-PE-labeled liposomes in normal mice was found to be almost identical to that of DOX-loaded liposomes. For example, 24 h post-injection the level of FITC-D-Lip was reduced to <0.1% of the ID, while levels of FITC-labeled SSL and SSIIL were >10% of the ID (data not shown).

Biodistribution of DOX and Its Metabolites in Tumor-Free Organs of Tumor-Bearing Mice

Time-dependent biodistribution of DOX and its total metabolites (DOX-M) was determined at 2, 6, and 24 h in the liver, spleen, kidney, and heart of mice injected iv with 35 μg DOX as D-SSL, D-SSIIL-IgG, or D-SSL-32/2. Organ distributions in tumor-bearing mice at tumor Stage I (micrometastases) and Stage II were almost identical. In all mice tested there was no effect of treatment on organ weight (see Fig. 2 legend). Only slight differences were found between the D-SSL, D-SSIIL-IgG, and D-SSL-32/2 regarding organ DOX levels, as demonstrated in Fig. 2 for mice with Stage II tumors. Only in spleen, the level of DOX delivered via the two D-SSL preparations was 1.5–2.0 times higher at all time points than that delivered by D-SSL, but this did not result in greater accumulation of DOX-M. For the three liposomal preparations the ratio of DOX-M to DOX in the liver was 3–5 times higher than in all other organs tested. In heart, for all three