Clinical Pharmacology of $^{99m}$Tc-labeled Liposomes in Patients with Cancer

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ABSTRACT

The pharmacokinetics, organ distribution, and 24-hr urinary excretion of negatively charged $^{99m}$Tc-labeled multilamellar liposomes, composed of dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol in a 7:3 molar ratio, were studied in seven patients with cancer. The radiolabeled liposomes were administered i.v. in three doses: 150 mg/sq m of body surface area; 300 mg/sq m; and 450 mg/sq m of lipid. The dose of $^{99m}$Tc was 4.8 to 7.6 mCi per patient. The plasma disappearance curve was biphasic (half-life $\alpha = 5.53$ min, half-life $\beta = 289$ min), suggesting a two-compartmental model of distribution. The calculated volume of distribution indicated considerable tissue retention of liposomes. This was confirmed by body imaging. Twenty-four hr after injection, liposomes were localized in organs rich in reticuloendothelial cells, i.e., liver [44.5 ± 9.1% (S.E.)], spleen [25.5 ± 7.7%], lung [14.5 ± 4.9%], and bone marrow. Although the hepatic uptake accounted for more than 40% of the total uptake, the spleen retained liposomes at a higher density. Cumulative urinary excretion of radioactivity was 13.4 ± 1.5% over 24 hr. Liposome administration was safe and devoid of any adverse side effects. The results provide a basis for the use of liposomes as potential target-specific and safe drug carriers in the treatment of pathological conditions that involve organs rich in reticuloendothelial cells.

INTRODUCTION

In the absence of a selective activity confined to a particular target organ or tissue, many drugs may cause undesirable side effects. Drug delivery systems designed for selective drug targeting may enhance the drug efficacy and may also decrease systemic toxicity, provided the organ selectivity for the drug uptake is compatible with localization in the diseased tissue. Since multilamellar liposomes are cleared by the RES (17), they may provide an ideal system for the delivery of drugs directed against pathological conditions in various parts of the RES. This approach has been successfully implemented using liposome:drug complexes for the treatment of several malignant (16, 18, 31) and infectious diseases in animal models (1, 7, 20, 21); for delivery of chemotherapeutic agents (6, 15), immuno-modulators (19), chelating agents (26), and enzymes (5); and for radiological and nuclear imaging (3, 9, 25). Liposomes offer a unique advantage over other delivery systems due to their biodegradability, ease of preparation, and lack of toxicity (13). Their distribution and pharmacokinetics can be modified by changing their size, charge, and lipid composition (14, 15). Clinical trials of liposomes as drug delivery systems in humans must be based on thorough Phase I pharmacology studies of empty liposomes, in order to provide essential information on the distribution, kinetics, and elimination of liposomes per se prior to their administration in a complex form with a specific drug. We report on the pharmacology of negatively charged $^{99m}$Tc-labeled multilamellar liposomes in 7 patients with cancer.

MATERIALS AND METHODS

Patients. Seven patients were entered in this study of the pharmacology of liposomes after informed consent was obtained according to institutional guidelines at the University of Texas-M. D. Anderson Hospital and Tumor Institute at Houston. Patient characteristics are summarized in Table 1. One patient with chronic myelogenous leukemia had had a splenectomy 16 months previously, and one patient with Hodgkin’s disease underwent unilateral nephrectomy 36 months before entering the study. Two patients with melanoma, one with chronic lymphocytic leukemia, and one with chronic myelogenous leukemia had active disease. The other 3 patients were in complete remission at the time of the study. None of the patients had received chemotherapy for at least 12 weeks prior to this study. Chest X-rays, complete blood counts, and liver and kidney function tests were performed prior to the study and at 48 hr after injection of liposomes. All patients were followed for at least 2 months after the study.

Lipids. Chromatographically pure (thin-layer chromatography solvents, CHCl{sub}3:CHOH::HNO{sub}3, 65:25:5) dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol used in this study were obtained from Avanti Polar Lipids (Birmingham, Ala.).

Preparation of Radiolabeled Liposomes. The entire labeling procedure was carried out in a laminar air flow hood using aseptic techniques. Initially, 10 to 13 mCi of $^{99m}$Tc-pertechnetate were reduced by the addition of 1.0 to 1.25 mg of 3 mM SnCl{sub}2 • 2H{sub}2O solution (prepared in nitrogen-purged sterile 0.9% NaCl solution) and incubation at room temperature for 5 to 10 min. The $^{99m}$Tc-labeled, negatively charged multilamellar vesicles were prepared as follows. The lipids dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol were added in a 7:3 molar ratio in a round-bottomed flask, and the solvent, chloroform, was evaporated to dryness using a rotary evaporator (Buchi; Brinkmann Instruments, Westbury, N. Y.). The residue was then dried in a vacuum desiccator for 20 min, and the radiolabeling was performed by the simultaneous addition of 5.0 ml of sterile 0.9% NaCl solution and a premeasured quantity of the stannous-reduced $^{99m}$Tc followed by an incubation of 20 to 30 min at room temperature. The flask was vortexed to disperse all the liposomes. Traces of colloidal tin and free pertechnetate were removed by washing the preparation twice with 5 to 7 ml of normal 0.9% NaCl solution and.
centrifuging at 30,000 \( \times \) g at 4°C for 15 min. The radioactivity in the pyrogen-free 0.9% NaCl solution. An aliquot of the suspension was kept liposomes remained at the origin, while the unbound pertechnetate labeling efficiency, which was consistently found to be 91 ± 3%. The content was quantitatively estimated by the analysis of the phosphorous lysate assay. Liposomes were sized in a Coulter Channelizer (Coulter Electronics, Hialeah, Fla.), and the particle size ranged between 0.2 and 5 \( \mu \)m in diameter, 67% being between 0.2 and 2 \( \mu \)m. The total lipid content was quantitatively estimated by the analysis of the phosphorous content as described previously (22). In vitro stability was assessed by incubating the preparation at 37°C in 100% serum for 2, 4, and 18 hr. The samples were centrifuged at 1800 \( \times \) g, and the pellet was washed twice with 0.9% NaCl solution. The radioactivity in the pellet obtained and in the supernatants was measured. It was found that more than 80% of the activity was still bound to the liposomes after 18 hr of incubation.

Liposome Administration and Pharmacokinetic Studies. The patients were divided into 3 groups according to total lipid doses. The first 3 patients received a total lipid dose of 150 mg/sq m of body surface area; 2 patients received 300 mg/sq m; and 2 patients received 450 mg/sq m. The liposome preparation was injected in a total volume of 10 ml over a period of 30 sec. Blood samples were drawn from a peripheral vein in the opposite arm at 1, 2, 3, 5, 10, 15, 20, 30, 60, 90, 120, and 240 min and at 24 hr following injection. After each withdrawal, the catheter was flushed with 0.9% NaCl solution containing 1% heparin. Whole blood (0.1 ml), plasma, and cell pellet samples were counted for radioactivity along with the control in an automatic gamma counter (Searle Analytic Model 1185). Pharmacokinetic parameters (volume of distribution, half-life (\( \alpha \) and \( \beta \)), clearance rate, and exposure duration were calculated by nonlinear regression analysis.

Nuclear Imaging Studies. A large-field \( \gamma \) camera (ON Model 410; Technicare Corp., Cleveland, Ohio) and scintillation data system (Gamma-1 system; Digital Equipment Corp., Iselin, N. J.) were also obtained after completion of the imaging.

Data Evaluation. Regions of interest in the lungs, liver, and spleen were defined on \( \gamma \) anterior images using a joy stick-directed cursor. Time activity curves for these regions and for the total activity in each image were then generated with standard software routines provided with the data system. The photographic density at manually selected points on the tomographic images was measured with a film densitometer (Model 301; X-Rite Co., Grand Rapids, Mich.).

RESULTS

Pharmacokinetics. The plasma pharmacokinetics of the labeled liposomes is summarized in Table 2. In all cases, regardless of dose, the plasma disappearance curve was biphasic and appeared to closely fit a 2-compartment mathematical model. There was no significant difference in the half-life or clearance rates for the 3 different lipid doses. The calculated volumes of distribution indicate a high tissue retention of liposome. Urinary excretion accounted for 13.4 ± 1.5% of the total injected dose at 24 hr.

Organ Distribution Studies. Organ distribution studies were performed at 3 time points (0.5, 4, and 24 hr). The data are shown in Table 3. While body scans showed that most of the radioactivity was in the liver, spleen, and lungs (Fig. 1), uptake was also seen in areas rich in bone marrow. Although the kidneys were visualized, their anatomical position precluded the establishment of a relative organ uptake. Distribution studies showed that, although the liver had the highest percentage of uptake of the total injected radioactivity, the concentration of liposomes in the spleen was higher at 4 hr than in the liver (spleen:liver ratios 1.32 ± 0.04; \( p < 0.01 \)). During the first 2 hr, there was detectable activity in the thyroid and urinary bladder. After 24 hr, these activities were no longer detectable. The kinetics of liposome uptake was studied in the liver, spleen, and lungs (Chart 1). Radioisotope concentration rose in these 3 organs during the first 3 min. Thereafter, the counts in the spleen were constant, while the counts in the liver continued to increase for 30 min before reaching a plateau. Lung activity was maximal after 2 min after injection. Chart 1 also shows the percentage of injected dose per ml of whole blood during the first 30 min after injection. These values were obtained by comparing blood activity to the activity in an aliquot of the control at the same time. A tricompartmental analysis of phagocytic activity was not possible due to the persistence of liposomes in these organs. Analysis of the plasma versus the cell pellet radioactivity showed that, during the first 4 hr after injection, there were no marked differences in the fractions (2933 cpm in the pellet to 3291 cpm in the plasma) and that, after 24 hr, the cell pellet was greater than that of plasma (1835 cpm in the pellet to 915 cpm in the plasma).

DISCUSSION

Understanding the \textit{in vivo} pharmacokinetic behavior and distribution of empty liposomes is an essential first step prior to their clinical use as drug carriers. Although the possibility exists that the drug:liposome complex may behave somewhat differently \textit{in vivo} from the carrier alone, the evidence available shows that the drug carried accumulates at the site of liposome uptake (15). In animal experiments, liposomes have been shown to decrease the toxicity associated with the administration of several antimicrobials and chemotherapeutic agents while enhancing their therapeutic efficacy (6, 18, 21, 23, 27, 29). The use of liposomal drug delivery systems in humans has been limited. Richardson et al. (28) studied the \textit{in vivo} localization of i.v.- administered liposomes in 14 patients with cancer without observing a preferential liposome uptake by neoplastic tissues. Several other trials have been conducted in humans: treatment of Gaucher’s disease (2); Pompe’s disease (30); respiratory distress syndrome in the newborn (24); rheumatoid arthritis (4); and hemophilia (10).

The pharmacokinetics of \textsuperscript{99m}Tc-labeled liposomes fits a 2-compartmental model of distribution. A calculated volume of
Liposome Pharmacokinetics in Cancer Patients

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/sq m)</th>
<th>Vₚ² (liter)</th>
<th>t₁/₂P (min)</th>
<th>C x f (µCi/ml x min)</th>
<th>Clearance rate (mL/kg x min)</th>
<th>24-hr urinary excretion (% of total dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>9.1</td>
<td>5.8</td>
<td>300</td>
<td>67</td>
<td>1.56</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>3.8</td>
<td>5.5</td>
<td>335</td>
<td>30</td>
<td>1.68</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>6.4</td>
<td>7.03</td>
<td>239</td>
<td>50.3</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>450</td>
<td>7.5</td>
<td>5.4</td>
<td>289</td>
<td>55.9</td>
<td>1.36</td>
</tr>
<tr>
<td>5</td>
<td>450</td>
<td>7.3</td>
<td>3.9</td>
<td>284</td>
<td>70.0</td>
<td>0.79</td>
</tr>
</tbody>
</table>

6.8 ± 0.86⁶, 5.53 ± 0.49², 289 ± 15², 54.6 ± 7.1², 1.27 ± 0.17², 13.4 ± 1.5²

⁶Vₚ, volume of distribution; C x f, exposure duration.
²Mean ± S.E.

Table 3

<table>
<thead>
<tr>
<th>Organ</th>
<th>% of uptake</th>
<th>30 min</th>
<th>4 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>48.4 ± 16.3⁶</td>
<td>44.5 ± 9.1</td>
<td>52.3 ± 11.7</td>
<td></td>
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<tr>
<td>Lung</td>
<td>20.1 ± 7.9 (0.3 ± 0.02)²</td>
<td>14.5 ± 4.9 (0.24 ± 0.07)</td>
<td>14.5 ± 5.2 (0.23 ± 0.15)</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>18.5 ± 10.6 (1.17 ± 0.2)²</td>
<td>25.5 ± 7.7 (1.32 ± 0.04)</td>
<td>19.4 ± 9.9 (0.91 ± 0.14)</td>
<td></td>
</tr>
</tbody>
</table>

⁶Mean ± S.E.
²Numbers in parentheses, uptake density ratio (photographic density ratio = photographic density of organ studied divided by photographic density of liver).

5 min following injection. Although the fecal excretion of the liposomes was not measured, the 24-hr urinary excretion accounted for less than 15% of the total dose, thus indicating the persistence of the liposomes in tissues. Similar to observations made in other animal species (3, 9, 25), more than 70% of the radioactivity at 0.5, 4, and 24 hr was present in reticuloendothelial cell-rich organs (liver, spleen, and lung). There was also uptake in the bone marrow and kidneys, but quantification of the isotope in these organs was not possible due to their anatomical position. The kinetics and tissue distribution of the liposomes did not seem to be markedly modified by the change in dose. When the ratio between plasma and peripheral blood cell radioactivity was assessed during several time points in the study, a relative increase in the cell pellet was observed over a 24-hr period. The reasons for these findings are not clear, but a plausible explanation is that liposomes are taken up and retained by circulating phagocytes. An interaction exchange of liposomal membrane lipids between cell liposome membranes and plasma lipoproteins is an alternate explanation.

The administration of liposomes was not associated with short- or long-term side effects, as assessed by clinical observation and chest X-rays, as well as by hematological, renal, and liver function tests.

The use of liposomal delivery systems in clinical trials in humans will depend upon the careful selection of drugs that, when entrapped in liposomes, will result in an increased therapeutic index. Due to the selective retention of liposomes in RES-rich organs, antimicrobial drugs directed against intracellular parasites and macrophage activators will be logical choices.

Specific targeting of liposome-entrapped anticancer drugs is not feasible at present; nevertheless, the potential for a reduced toxicity of these agents may well be a reason to use these carriers in cancer therapy. New developments in liposome technology such as combination of liposomes with hyperthermia, coupling of target-specific antibodies to liposomes, magnetic localization, RES blockade, and manipulation of the liposome physicochemical characteristics will undoubtedly increase the...
potential of liposomes as drug carriers in the therapy of human diseases.

REFERENCES

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