Distribution of Technetium-99m-Labeled Multilamellar Liposomes in Patients with Hodgkin's Disease

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The distribution of ^{99m}Tc-labeled multilamellar liposomes composed of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) at a molar ratio of 7:3, administered intravenously, was studied in ten patients with Hodgkin's disease (HD). The dose of lipid was 150 mg/m^2 and the mean dose of radioactivity injected per patient was 8.1 mCi (range 6.7–9.8). Whole-body imaging techniques were used, and for each organ an uptake index was calculated as the percent photographic density (PD) relative to the PD of the liver. Results were compared to those in a group of six patients with other malignancies. Increased liposome uptake in several skeletal areas was observed in one patient with HD with diffuse bone involvement and in the bone marrow of two patients with HD with bone marrow involvement. No definite liposome uptake was observed in lymph nodes involved by HD or in tumor areas of patients with other malignancies. Patients with HD had a significantly higher uptake by bone marrow (23.8% compared with 10.2% at 4 hr $p = 0.02$), and lungs (59.6% compared with 25.0% at 4 hr, $p = 0.01$) than patients with other malignancies. Among patients with HD, the uptake by bone marrow and lungs were higher in those with constitutional symptoms (bone marrow at 4 hr 31.4% compared with 16.2%, $p = 0.02$; lungs at 4 hr 68.8% compared with 50.4%, $p = 0.19$) and with liver involvement (bone marrow at 4 hr 30.8% compared with 16.8%, $p = 0.03$; lungs at 4 hr 73.6% compared with 45.6%, $p = 0.03$). These results suggest that patients with HD have a different pattern of distribution of multilamellar liposomes which may be related to a combination of nonspecific stimulation of the reticuloendothelial system and tumor uptake. It does not appear that liposomal ^{99m}Tc is capable of adequately imaging HD for clinical diagnosis.

J Nucl Med 26:743–749, 1985

Liposomes are lipid vesicles with a bilayer structure that form spontaneously upon the addition of aqueous solutions to dried lipid films (*1–3*). In humans, when liposomes are injected intravenously, they are rapidly cleared from the circulation by the reticuloendothelial system (RES) of the liver, spleen, and to a lesser degree, the lung (*1–4*). The rate of RES clearance depends on the composition, charge, and size of the particles. Multilamellar vesicles (size range: 0.2–5 μm) are mainly cleared by the RES by phagocytosis, while unilamellar vesicles (size range: 0.05–0.1 μm) are also significantly taken up by hepatocytes (*3*).

Hodgkin's disease (HD) is a malignant neoplasm composed of giant multinucleated cells (Reed-Sternberg cells) and a mixture of normal inflammatory cells (T-lymphocytes, monocytes, and eosinophils). Reed-Sternberg cells constitute the malignant cells of the HD lesions (*5*). However, they account for less than 1% of the tumor mass (*6*). Some studies have shown that Reed-Sternberg cells have in vitro morphologic and functional characteristics of the monocyte-macrophage

Received Nov. 5, 1984; revision accepted Mar. 13, 1985.

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lineage (*7–9*), including a variable but definite phagocytic capacity for latex beads, iron particles, red blood cells, and agar. No in vivo assessment of the phagocytic capacity of Reed-Sternberg cells is available although the presence of intracytoplasmic polyclonal immunoglobulin has been previously observed (*10*).

Compared with the various colloidal preparations used for diagnostic purposes in nuclear medicine, liposomes have the advantage of being potential drug carriers and, therefore, a promising tool in the treatment of infections and neoplastic diseases that involve or arise in the RES (*11–13*).

In the present study, we have analyzed the distribution of technetium-99m- (^{99m}Tc) labeled multilamellar liposomes given intravenously to a group of patients with HD in order to investigate the potential of liposomes as a diagnostic tool and carriers of anticancer agents in this disease.

MATERIALS AND METHODS

Patients

Ten patients (eight males and two females) with active HD were included in the study. None had received chemotherapy or radiotherapy for at least 6 wk prior to the study. The mean age was 31 yr (range 22–46 yr). Seven had nodular sclerosing, and three had mixed cellularity HD. Six were previously untreated (two stage II, one stage III and three stage IV) and four were studied at the time of relapse. Five had constitutional symptoms (noninfectious fever, night sweats, or weight loss). Four had undergone splenectomy.

The prestudy workup consisted of physical examination, chest x-ray, bone marrow biopsy, computerized tomographic (CT) scan of the abdomen, and lymphangiogram. In addition, four patients also had a bone scan performed and two a liver-spleen scan. Liver and spleen involvement were classified as diffuse if there was only a homogeneous organ enlargement seen by CT scan and/or liver-spleen scan or focal if there was a nonhomogeneous enlargement or the presence of cold areas. Eight patients had lymph node involvement (one of <3 cm, four of 3–7 cm and three of >7 cm), five had liver involvement (three nodular and two diffuse) and two had spleen involvement. Two patients had bone marrow involvement and two other patients bone disease based on abnormalities in the bone scan and the presence of hypercalcemia. One patient had bilateral pulmonary nodules.

Six patients with miscellaneous neoplasms (two with melanoma, three with chronic leukemia, and one with oat cell carcinoma of the lung) were used as a control group. All patients had active disease except one with chronic lymphocytic leukemia and the patient with oat cell carcinoma of the lung. None of the patients in the control group had received chemotherapy for at least 12 wk prior to the study. No patient in the control group had liver involvement.

An informed consent was obtained in all patients according to institutional guidelines.

Preparation of radiolabeled liposomes

Radiolabeled multilamellar liposomes were prepared using a standardized procedure modified from Morgan et al. (*14*) as described previously (*4,15*). Briefly, a mixture of dimyristoyl phosphatidylcholine (DMPC)* and dimyristoyl phosphatidylglycerol (DMPG)* at a molar ratio of 7:3 in chloroform was added to a round bottom flask and the solvent was evaporated to dryness with nitrogen gas. Subsequently, the radiolabeling was performed by the simultaneous addition of premeasured amounts of sterile normal saline and stannous-reduced ^{99m}Tc. After an incubation of 20 min at room temperature, the flask was vortexed to disperse all the liposomes. Free pertechnetate, traces of colloidal tin, and the smaller sized vesicles were removed by centrifugation at 30,000 × *g* for 10 min. The liposome rich residue containing the larger-sized vesicles was washed twice with normal saline and centrifuged at 30,000 × *g* for 5 min. The total activity in the supernatants and residue was counted to estimate the percent yield of labeled liposomes. The labeling efficiency (percent of tracer entrapped in liposomes) was 90 ± 2%. Liposomes were sized in a Coulter Channelizer and the particle size ranged between 0.2 and 5 μm in diam, 67 ± 5% being between 0.2 and 2 μm. The sterility of the radiolabeled preparation was estimated in a Bactec growth detector† and the endotoxin content was determined by a Limulus amebocyte lysate assay.

Liposome administration

The total amount of lipid administered per patient was 150 mg/m^2 of body surface area. The liposome preparation was injected intravenously in a total volume of 5–10 ml over a period of 30 sec. The mean radioactivity injected per patient was 8.1 mCi (range 6.7–9.8). All 16 patients were studied once except one patient with HD, who was studied in complete remission and in relapse. No side effects were observed after liposome ^{99m}Tc administration.

Nuclear imaging studies

A large-field gamma camera and scintillation data system were used to record the distribution of radioactivity in the thorax and upper abdomen during a period of 30 min beginning at the time of injection. Static images of anterior and posterior projections were recorded at 10, 20, and 30 min after injection. Total-body longitudinal tomographic images were obtained at 4 and 24 hr after injection.

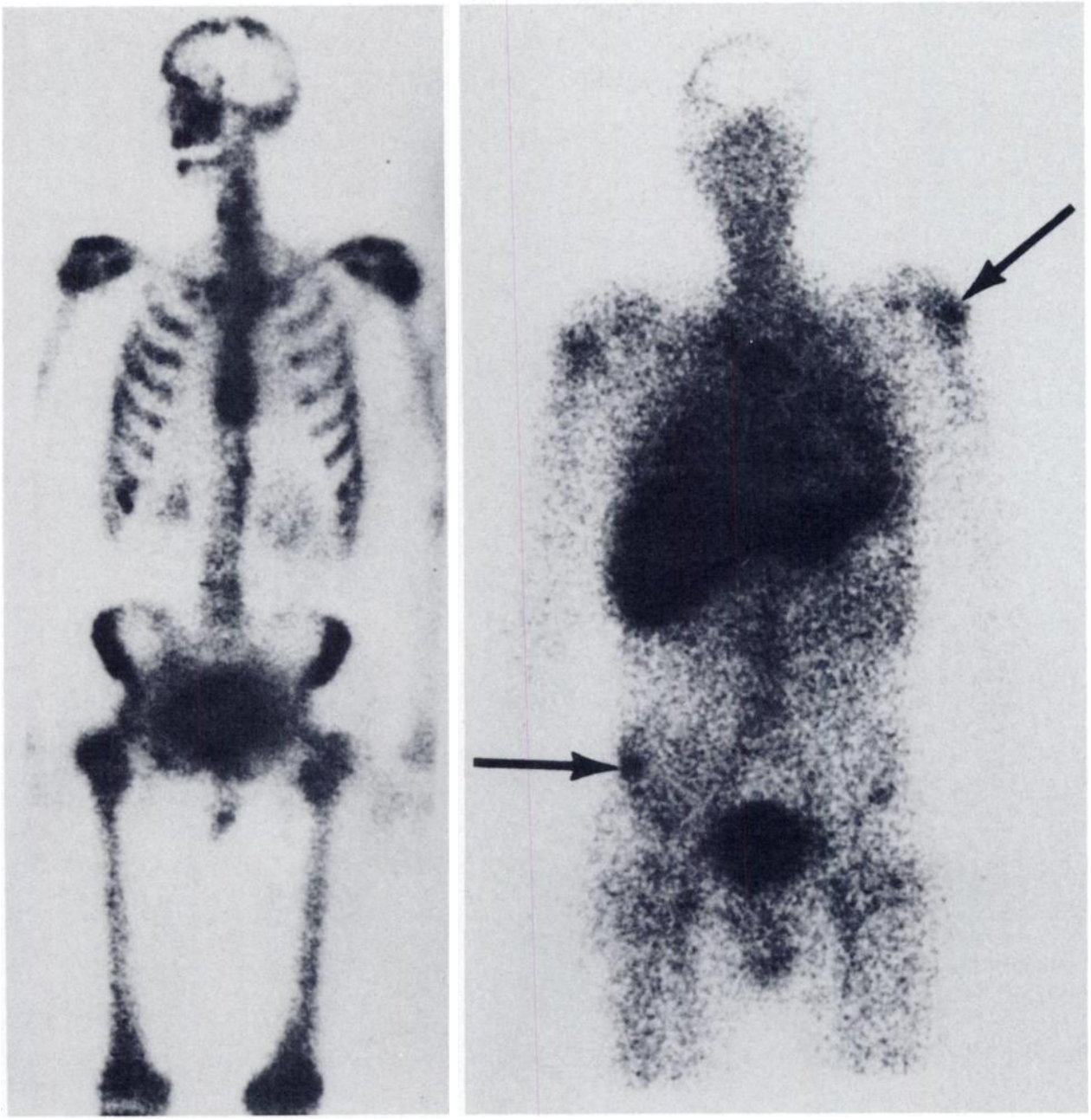

FIGURE 1
Tomographic view of ^{99m}Tc liposome scan (right) of patient with HD disease with diffuse bone involvement. Increased liposome uptake is evident in right pelvis and left humerus (arrows). Bone scan (left) shows several spots of increased activity in pelvis, chest, spine, and extremities

Data evaluation

The photographic density (PD) of the liver, spleen, kidney, lungs, and bone marrow on the anterior and posterior tomographic images was measured with a film densitometer. The PD of the bone marrow was measured in the pelvic and sacroiliac areas with a higher liposome uptake. A liposome uptake index was calculated for each organ using the mean PD values of both tomograms according to the following formula: PD organ/PD liver × 100. The liver was chosen as the reference organ since the spleen was absent in four patients in the group with HD and in one patient in the control group. In the patients who had not had splenectomy, the uptake index for the liver was calculated as: PD liver/PD spleen × 100. All measurements of PD were made by one of the authors (TPH) without knowing the patients diagnosis.

Statistical analysis

The differences in liposome uptake by organs between

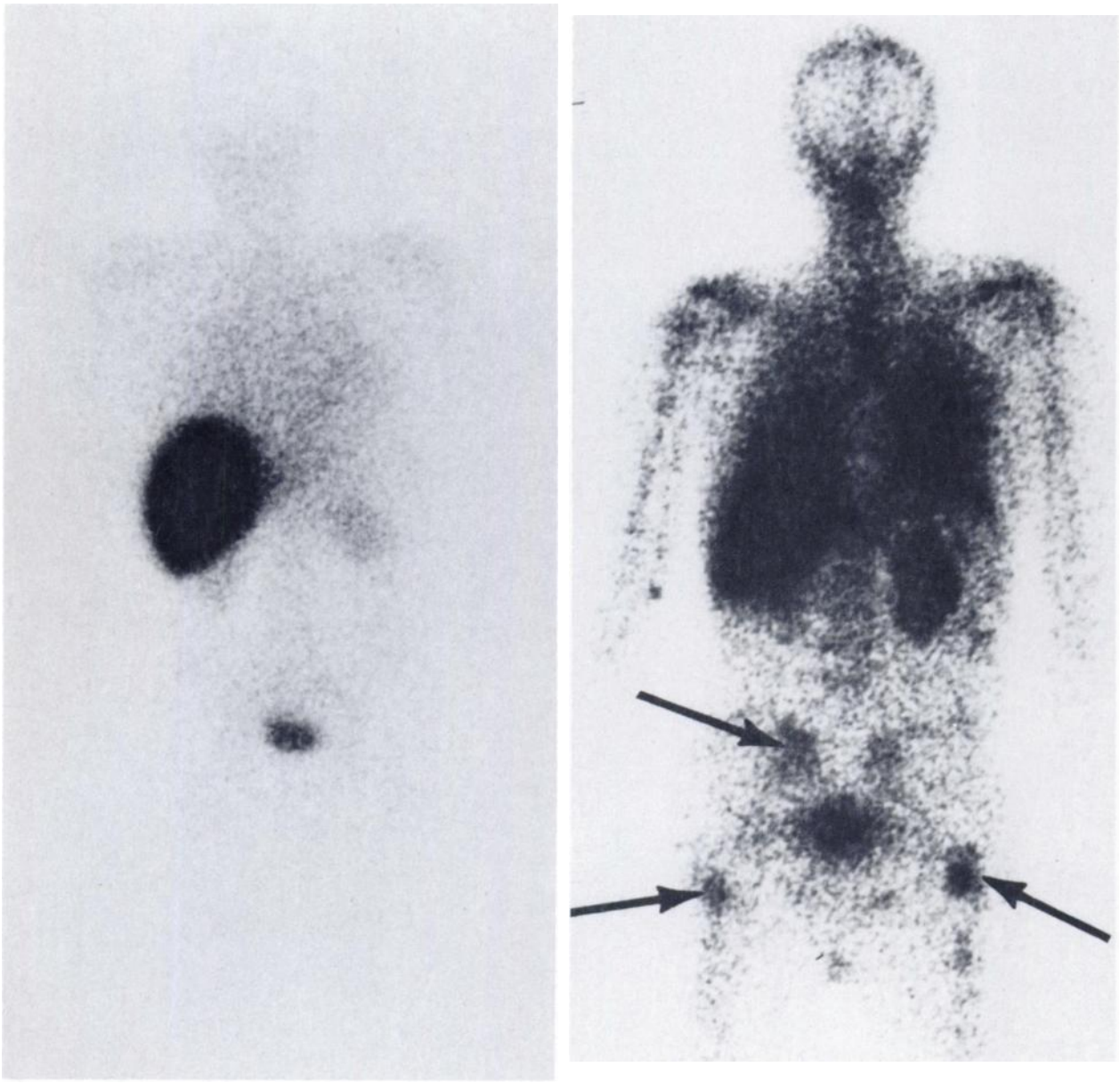

FIGURE 2
Tomographic view of ^{99m}Tc liposome scan of same patient as in Fig. 1 (right) and patient with chronic myelogenous leukemia (left). Both patients had bone marrow involvement and had been previously splenectomized. Liposome uptake by bone marrow and lungs is increased in patient with HD. Noted again are several areas of increased liposome uptake in pelvis and femurs of patient with HD (arrows)

groups of patients were analyzed for statistical significance using a t-test. All p values are of the two-sided type.

RESULTS

Tumor localization of ^{99m}Tc-labeled liposomes

Several skeletal areas with an increased concentration of ^{99m}Tc-labeled multilamellar liposomes were observed in one patient with HD who had diffuse bone involvement (Figs. 1 and 2). These areas were also detected by a [^{99m}Tc]sulfur colloid scan. Both patients with HD and bone marrow involvement had higher liposome uptake by bone marrow than patients without bone marrow involvement (30.0% compared with 22.2% at 4 hr and 17.5% compared with 11.9% at 24 hr). These two patients also had liver disease, but their liposome uptake by bone marrow was still higher than that of the three other patients with HD and liver involvement (17.5% compared with 13.3% at 24 hr).

Liver involvement by HD was focal in three of five

TABLE 1
Liposome Uptake by Different Organs in Patients with Hodgin's Disease and Other Malignancies

Organ	Time of scan (hr)	Uptake index (mean ± s.d.)* Hodgkin's disease	Other malignancies	p
Spleen	4	111.0 ± 17.8	123.5 ± 27.5	0.40
	24	109.5 ± 26.3	160.4 ± 117.0	0.32
Liver	4	91.7 ± 12.4	84.5 ± 21.6	0.52
	24	94.5 ± 18.0	84.8 ± 45.3	0.64
Lung	4	59.6 ± 21.5	25.0 ± 22.5	0.01
	24	28.3 ± 16.9	14.7 ± 16.2	0.13
Bone marrow	4	23.8 ± 11.1	10.2 ± 5.5	0.02
	24	13.9 ± 6.2	7.5 ± 3.6	0.04
Kidney	4	26.0 ± 10.7	16.0 ± 8.5	0.11
	24	28.0 ± 7.1	23.3 ± 12.2	0.41
Bladder	4	75.2 ± 35.8	65.8 ± 62.2	0.71
	24	21.1 ± 14.0	13.0 ± 9.0	0.23

* Uptake index = PD organ/PD liver × 100.

patients. In two, concomitant radionuclide liver-spleen scans were available for comparison. The pattern of liver distribution of ^{99m}Tc-labeled multilamellar liposomes was almost identical to that of ^{99m}Tc colloid with several relatively cold areas surrounded by normal parenchyma. In the two patients with diffuse liver disease, the liposome uptake by the liver was normal.

Of eight patients who had lymph node involvement, only in one who had a large mediastinal mass was there some evidence of radioactivity in the tumor area. However, it was not possible to assess how much of the presumable liposome uptake by tumor corresponded to the spine. In all the other cases, no definite increased uptake in the areas of lymph node involvement was observed. No consistent relationship between the histologic type of HD and the presence of liposome uptake in tumor areas was observed. No liposome uptake by tumor was observed in the patients with other malignancies.

Organ distribution of ^{99m}Tc-labeled liposomes

The liver and spleen were the organs with the highest liposome uptake both in the control group, and in the patients with HD (Table 1). The analysis of liposome uptake by the different organs showed a significant increase in liposome uptake by bone marrow both at 4 hr (23.8% compared with 10.2%, p = 0.02) and 24 hr (13.9% compared with 7.5%, p = 0.04) and in liposome uptake by the lungs at 4 hr (59.6% compared with 25.0%, p = 0.01) in patients with HD compared with patients with other malignancies. The same trend was found in patients with HD and no bone marrow or lung involvement. The differences in liposome uptake by the spleen, kidney and bladder were not significant (Table 1). The bladder uptake was most likely secondary to glomerular filtration of the smaller liposomes and free ^{99m}Tc detached from liposomes in the plasma.

In patients with HD, liposome uptake by the different organs was analyzed according to the presence of constitutional symptoms, untreated compared with relapsing disease and liver involvement. Liposome uptake by bone marrow was significantly increased at 4 hr in patients with constitutional symptoms compared with patients without constitutional symptoms (31.4% compared with 16.2%, p = 0.02) and patients with liver involvement compared with patients without liver involvement (30.8% compared with 16.8%, p = 0.03) (Table 2). The same trend was observed at 24 hr. Four of five patients with liver involvement had constitutional symptoms.

Liposome uptake by the lungs was significantly higher in patients with liver involvement than in patients without liver involvement both at 4 hr (73.6% compared with 45.6%, p = 0.03) and 24 hr (38.4% compared with 18.2%, p = 0.05). Liposome uptake by the lungs also tended to be higher in patients with constitutional symptoms than in patients without these symptoms both at 4 hr and 24 hr but the differences did not reach statistical significance (Table 2). No differences in liposome uptake by the spleen, kidney and bladder were seen between the different subgroups of patients with HD. No differences in organ distribution were observed between patients with untreated HD and patients with HD in relapse.

In the patient who was studied both while in complete

TABLE 2
Liposome Uptake by Different Organs According to the Presence of Constitutional Symptoms and Liver Involvement

Organ	Constitutional symptoms (uptake index*) Present	Absent	p	Liver involvement (uptake index*) Yes	No	p
Spleen	105.2	122.5	0.31	104.3	117.7	0.42
Liver	95.2	84.5	0.37	96.0	87.8	0.45
Lung	68.8	50.4	0.19	73.6	45.6	0.03
Bone marrow	31.4	16.2	0.02	30.8	16.8	0.03
Kidney	37.8	21.4	0.06	30.5	24.2	0.53

* Uptake index = PD organ/PD liver × 100.

remission and with active HD, the presence of active disease was associated with increased liposome uptake by the bone marrow and lungs both at 4 hr and 24 hr (uptake by lungs at 4 hr 45.0% compared with 29.8% when in complete remission, uptake by bone marrow at 4 hr 42.0% compared with 12.5% when in complete remission). This patient had diffuse liver involvement but no spleen involvement. No major differences were seen in liposome uptake by the other organs.

DISCUSSION

The results obtained demonstrate an increased liposome uptake by several skeletal areas in one HD patient with diffuse bone involvement and in both HD patients with bone marrow involvement. The identification of the tissue compartment responsible for the increased liposome uptake in these areas is not possible with the imaging techniques used and would require tissue autoradiographic studies.

Nodular liver lesions by HD were detected in the liposome scans as relatively cold areas. This indicates that their phagocytic activity is lower than that of the normal liver parenchyma, which is, after the spleen, the organ with the highest phagocytic activity. No definite liposome uptake was observed in lymph nodes involved by HD. Lymph nodes are rich in RES cells and when multilamellar liposomes are administered intralymphatically (Perez-Soler R, unpublished data) they immediately concentrate in the lymph nodes encountered. However, when given systemically, due either to the low blood flow or the endothelial structure, the liposome uptake by lymph nodes is minimal. The use of small liposomes (SUV) which have a longer serum half-life might enhance the liposome uptake by the lymphatic system (*2*).

Patients with HD, especially those with constitutional symptoms and liver involvement, had an increased liposome uptake in the bone marrow and lungs compared with patients with other malignancies. Since four of five patients with liver involvement also had constitutional symptoms, it is not possible to discern which factor was directly related to this different pattern of liposome distribution.

Liposome uptake by phagocytic Reed-Sternberg cells might explain the increased liposome uptake by the skeleton in the patients with HD with bone or bone marrow involvement. However, since Reed-Sternberg cells constitute a very small tissue compartment in HD lesions (less than 1%), it is very unlikely that liposome uptake accounts entirely for the increased uptake observed in those areas. A second mechanism could be uptake by normal stimulated phagocytic cells of the monocyte-macrophage lineage since HD is associated with a complex immune-dysregulation in which the monocyte-macrophage lineage probably plays an important role (*16*). The RES phagocytic capacity for iodine-125-labeled albumim and the production of PGE_2 by peripheral monocytes are increased in patients with HD (*17,18*). The increased liposome uptake in lungs and bone marrow observed in HD patients might be a manifestation of the stimulation of the RES suggested by these studies. This would also explain the correlation between the increased uptake by bone marrow and lungs and the presence of constitutional symptoms, since these symptoms may be related to stimulation of the monocyte-macrophage system. Finally, the differences in liposome distribution might be a reflection of differences in the pattern of organ involvement rather than tumor type. Five of ten patients with HD had liver involvement versus none of six with other malignancies. The presence of liver involvement may result in an increased liposome availability to other organs rich in RES cells. However, patients with HD without liver involvement had still a higher liposome uptake by bone marrow and lungs than patients with other malignancies, (bone marrow at 4 hr: 16.8% compared with 10.2%, lungs at 4 hr: 45.6% compared with 25.0%). The amount of lipid administered was 150 mg/m^2 in all patients with HD. Doses of up to 450 mg/m^2 of the same type of liposome preparation do not result in a different pattern of liposome distribution (*4*), which suggests that the dose administered was well below the saturation level of the normal RES.

The present study provides evidence that the pattern of distribution of multilamellar liposomes in patients with HD is different from that of patients with other malignancies. The difference is most likely secondary to the stimulation of the RES associated with HD. It does not appear that liposomal ^{99m}Tc is capable of adequately imaging HD for clinical diagnosis but it is too early to state whether this different distribution pattern can be exploited for therapeutic purposes. Autoradiographic studies are necessary to assess the uptake of liposomes by tumor tissue and therefore to provide a basis for the use of anticancer agents entrapped in liposomes in the treatment of refractory HD.

FOOTNOTES

* Avanti Polar Lipids, Birmingham, AL.

† Model 301, Johnston Laboratories, Inc., Cockeysville, MD.

ACKNOWLEDGMENTS

The authors thank Jennie Rayls for preparing the manuscript.

This work was supported in part by Grants CA 31544 and BRSG 5511 from the National Cancer Institute, National Institutes of Health, Bethesda, MD 20205. Dr. Lopez-Berestein is a recipient of a Fellowship from the Leukemia Society of America.

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