Preclinical and Clinical Evidence for Disappearance of Long-Circulating Characteristics of Polyethylene Glycol Liposomes at Low Lipid Dose

PETER LAVERMAN, ADRIENNE H. BROWERS, ELS TH. M. DAMS, WIM J. G. OYEN, GERT STORM, NICO VAN ROOIJEN, FRANS H. M. CORSTENS, and OTTO C. BOERMAN

Department of Nuclear Medicine, University Medical Center Nijmegen, Nijmegen, the Netherlands (P.L., A.H.B., E.Th.M.D., W.J.G., F.H.M.C., O.C.B.); Utrecht Institute for Pharmaceutical Science, Utrecht University, Utrecht, the Netherlands (G.S.); Department of Cell Biology and Immunology, Faculty of Medicine, Free University, Amsterdam, the Netherlands (N.v.R.)

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ABSTRACT

This study describes the effect of the lipid dose of $^{99m}$Tc-polyethylene glycol (PEG) liposomes in the low-dose range (0.02–1.0 $\mu$mol/kg) on the pharmacokinetics and biodistribution in rats, rabbits, and humans. The biodistribution and pharmacokinetics of $^{99m}$Tc-PEG liposomes at various dose levels were studied in rats and rabbits with a focal Escherichia coli infection. Scintigraphic images were recorded on a gamma camera. In addition, the role of macrophages in the biodistribution of a low-dose PEG liposome injection was studied. Finally, the pharmacokinetics of $^{99m}$Tc-PEG liposomes at two lipid dose levels was studied in four patients. At a dose level of 0.03 $\mu$mol/kg, the blood level in rats at 4 h postinjection was significantly lower than at the highest dose level (1.1 $\mu$mol/kg). The same effect was observed in rabbits where enhanced clearance was observed at a dose level of 0.02 $\mu$mol/kg. The circulatory half-life decreased from 10.4 to 3.5 h (at 1.0 and 0.02 $\mu$mol/kg, respectively). At the lowest dose level, liposomes were mainly taken up by the liver and to a lesser extent by the spleen. Injection of a low dose of PEG liposomes in macrophage-depleted rabbits resulted in normal pharmacokinetics, suggesting involvement of macrophages in the effectuation of the rapid elimination of the liposomes from the circulation. Most importantly, the rapid clearance of low-dose PEG liposomes was also observed in humans when relatively low lipid doses were administered. This study showed that at very low lipid doses the biodistribution of PEG liposomes is dramatically altered.

Soon after the discovery of liposomes, it was recognized that liposomes labeled with $\gamma$-radiation-emitting radionuclides could be used as radiopharmaceuticals to visualize pathological processes, such as infections and tumors, in vivo. However, liposomes in their classical composition were less suitable because of their short circulatory half-life and high uptake in liver and spleen. The circulatory half-life of these so-called “conventional” liposomes could be enhanced by reducing their size, giving them a positive surface charge, and including phospholipids with saturated acyl chains (Gorgoriadis et al., 1974; Juliano and Stamp, 1975). In addition, it was shown that the blood residence time of conventional liposomes increases with the lipid dose given. Beaumier et al. (1983) showed in mice that small liposomes (~50 nm) at 6 mg of liposomal lipid/kg b.wt. had relatively high liver uptake [60%ID (percentage of the injected dose), 23 h postinjection (p.i.)], whereas at doses exceeding 120 mg/kg b.wt., liver uptake was much lower (20%ID, 23 h p.i.). The blood clearance pattern in these experiments virtually mirrored the liver uptake. These experiments demonstrated that the liver uptake mainly resulted from saturable phagocytosis by Kupffer cells.

The development of liposomes coated with the hydrophilic polymer polyethylene glycol (PEG) greatly broadened the application of liposomes (Woodle and Lasic, 1992). PEG-coated liposomes (also known as stealth liposomes) are considered to have long-circulating properties irrespective of lipid composition, surface charge, and/or lipid dose. In various preclinical studies, as well as in a clinical study, we have shown that PEG liposomes labeled with $^{99m}$Tc-hexamethylpropylene-amine-oxime performed well in visualizing various types of infection and inflammation (Boerman et al., 1997; Dams et al., 1998, 1999, 2000). The development of an improved labeling method, based on the bifunctional chelator hydrazinonicotinamide (HYNIC), enabled us to reduce the administered lipid dose to 20% of the initially used dose (Laverman et al., 1999). Recently, however, Utkhede and

ABBREVIATIONS: %ID, percentage of injected dose; PEG, polyethylene glycol; p.i., post injection; MPS, mononuclear phagocyte system; HYNIC, hydrazinonicotinamide; DSPE, distearoylphosphatidyl-ethanolamine; PHEPC, partially hydrogenated egg phosphatidyl choline.
Tilcock (1998) showed that the circulation time of PEG liposomes at very low lipid dose levels was strongly reduced, whereas uptake in the liver and the spleen was clearly enhanced. To further elucidate this phenomenon, we investigated in detail the effect of lipid dose on the pharmacokinetics and biodistribution of PEG liposomes in two animal species as well as in humans.

In both rats and rabbits with an artificially induced focal infection, we determined the pharmacokinetics and the biodistribution of radiolabeled PEG liposomes at various lipid dose levels. Furthermore, we investigated the effect of two successive injections of PEG liposomes at low lipid dose. In rabbits we also studied the effect of macrophage depletion on the blood clearance of liposomes at low lipid dose. Finally, in four patients the blood clearance and biodistribution of PEG liposomes at two lipid dose levels was assessed.

Materials and Methods

Reagents

Partially hydrogenated egg-phosphatidylethanolamine (PHEPC) with an iodine value of 35 was a gift from Lipoid GmbH (Ludwigshafen, Germany). Distearoylphosphatidylethanolamine (DSPE) and the PEG-2000 derivative of DSPE were purchased from Avanti Polar Lipids (Alabaster, AL). N-Hydroxysuccinimidyld-hydrazinonicotinamide hydrochloride (S-HYNIC) was synthesized as described by Abrams et al. (1991) with minor modifications (Claessens et al., 1996). The hydrazinonicotinamide derivative of DSPE (HYNIC-DSPE) was prepared as described previously (Laverman et al., 1999). Clodronate (Cl2MBP) was a gift from Boehringer Mannheim GmbH (Mannheim, Germany).

Liposome Preparation

PEG liposomes were prepared as described previously (Laverman et al., 1999). Briefly, a lipid mixture in methanol/chloroform (10:1) was prepared with a molar ratio of 1.85:0.15:0.07:1 (PHEPC/PEG-DSPE/HYNIC-DSPE/cholesterol) unless stated otherwise. After evaporation of the organic solvents, the resulting lipid film was dispersed in HEPES buffer [10 mM HEPES (ICN Biomedicals, Inc., Costa Mesa, CA), 135 mM NaCl at pH 7.4] at room temperature. Liposomes were sized by multiple extrusion through two stacked polycarbonate membranes with a medium pressure extruder (Lipex Biomembranes, Inc., Vancouver, BC). After sizing, the suspension was dialyzed against HEPES buffer overnight at 4°C with four buffer changes to remove unconjugated S-HYNIC. Liposomes were stored in HEPES buffer at 4°C.

Mean particle size of the liposomal preparations was determined by a dynamic light scattering system (Malvern 4700; Malvern Instruments Ltd., Malvern, UK). The mean size of the liposome was 90 nm, with a polydispersity index of 0.1. This index ranges from 0.0 for an entirely monodisperse dispersion to 1.0 for a completely polydisperse dispersion. Phospholipid concentration in the liposome dispersion was determined using the colorimetric method described by Rouser et al. (1970).

Radiolabeling

Labeling of the liposomes was performed as described earlier (Laverman et al., 1999) with minor modifications. Briefly, various volumes of liposomes (85 µmol of phospholipid) were adjusted to a final volume of 100 µl with HEPES buffer and added to a mixture of 10 mg of Tricine, 10 µg of stannous sulfate, and 99mTcO4− in saline. The mixture was incubated for 20 min at room temperature. Radiochemical purity was determined using instant thin-layer chromatography on silica gel strips (Gelman Sciences, Inc., Ann Arbor, MI) with 0.15 M sodium citrate buffer, pH 5.5, as the mobile phase. When radiochemical purity was less than 95%, liposomes were further purified by gel filtration on a PD-10 column with HEPES buffer as the eluent.

Animal Experiments

Rat Model of Infection. An abscess was induced in the left calf muscle of young, male, randomly bred Wistar rats (220–240 g) with approximately 1 × 109 colony-forming units of Escherichia coli in 0.1 ml of a 1:1 suspension of autologous blood and normal saline. During the procedure, animals were anesthetized with ether. After 24 h, when swelling of the muscle was apparent, the radiolabeled liposomes were injected i.v. via the tail vein.

Rabbit Model of Infection. An abscess was induced in the left thigh muscle of female New Zealand White rabbits (2.2–2.8 kg) with approximately 1.5 × 109 colony-forming units of E. coli in 0.5 ml of normal saline. During the procedure, animals were anesthetized with a 0.6-ml s.c. injection of a mixture of fentanyl 0.315 mg/ml and flunisone 10 mg/ml (Hynorm; Janssen Pharmaceuticals, Buckinghamshire, UK). After 24 h, when swelling of the muscle was apparent, the radiolabeled liposomes were injected i.v. via the lateral ear vein.

Gamma Camera Imaging and Biodistribution Studies. Twenty-four hours after E. coli inoculation, groups of five rats received 10 MBq 99mTc-PEG liposomes via the tail vein. Rabbits (five per group) were injected with 15 MBq 99mTc-PEG liposomes via the lateral ear vein. From each group, at least three animals were placed prone on a gamma camera (Orbiter; Siemens Medical Systems, Inc., Hoffman Estates, IL) equipped with a parallel-hole low-energy collimator, and images were recorded at 5, 30, 60, 120, and 240 min p.i. After acquisition of 100,000 counts, the images were stored digitally in a 256 × 256 matrix.

Blood samples from the rabbits were collected at 1, 30, 60, 120, and 240 min after injection of the radiopharmaceutical to determine the blood clearance of the radiolabeled liposomes. Data were analyzed by nonlinear least-squares fit using a biexponential model.

Effect of Macrophage Depletion. To study the involvement of macrophages on the in vivo behavior of PEG liposomes, liver and splenic macrophages in rabbits were depleted by injection of multilamellar liposomes containing dichloromethylene-bisphosphonate (Cl2MBP) or clodronate. Clodronate liposomes and PBS-containing liposomes (serving as control) were prepared as described previously by Van Rooijen and Sanders (1994). Two female New Zealand White rabbits (2.0–2.5 kg) were injected i.v. with 4 ml of clodronate liposomes (containing approximately 20 mg of clodronate), and two rabbits were injected with 4 ml of PBS liposomes. After 48 h, when depletion of the macrophages in liver and spleen was most optimal (Van Rooijen and Sanders, 1994), all rabbits were injected i.v. with 10 MBq 99mTc-PEG liposomes (0.02 µmol/kg). Gamma camera imaging was acquired at 5 min, 2, and 4 h p.i. After the final images were recorded, rabbits were sacrificed, tissues were dissected and the biodistribution of the radiolabel was determined as described above. Parts of liver and spleen were snap-frozen in dry ice-cooled isopentane and stored at −80°C. Cryostat sections were stained for acid phosphatase to confirm macrophage depletion (Zysk et al., 1997).

Clinical Studies

Four patients (two males, two females, ages 45, 56, 57, and 64 years), referred to the Department of Nuclear Medicine for imaging of infection and inflammation, were included in our study. Informed
consent was obtained from each patient. The study protocol had been approved by the ethical review board of the University Hospital Nijmegen. Three patients were suspected of infection or inflammation of the musculoskeletal system, and one patient had possible abdominal pathology (Table 1).

All four patients received 740 MBq 99mTc-PEG liposomes (0.5 or 0.1 μmol/kg b.wt.) and were imaged at 4 and 24 h p.i. of the radiopharmaceutical. Scintigraphic images were obtained with a Multi-Spect-2 gamma camera connected to an ICON computer system (Siemens Inc., Hoffman Estates, IL). All images were collected in digital format in a 256 × 256 matrix. Whole body scans were recorded at 4 and 24 h p.i., scan speed 15 and 6 cm/min, respectively. The scintigraphic results were analyzed by drawing regions of interest over the liver and the spleen. An injection standard was recorded simultaneously to permit the calculation of the %ID in both organs.

Blood samples were collected at 0, 5, 15, 30 min, and 1, 2, 4, and 24 h after injection of the liposomes. The samples were weighed, and radioactivity was counted and expressed as %ID. Data were analyzed by nonlinear least-squares fit using a biexponential model.

**Statistical Analysis**

All mean values are expressed as mean ± S.D. Statistical analysis was performed using the one-way ANOVA and corrected for multiple comparisons by the Bonferroni procedure. The level of significance was set at P < .05.

**Results**

The lipid dose dependence of the biodistribution of 99mTc-PEG liposomes was studied in rats with an E. coli infection in the left calf muscle. Three groups of five rats received 99mTc-PEG liposomes either at 1.1, 0.4, or 0.03 μmol of phospholipid/kg b.wt. As shown in Table 2, at the lowest lipid dose (0.03 μmol/kg), the blood level of the 99mTc-PEG liposomes was significantly lower as compared with the blood level after injection of the highest lipid dose of 1.1 μmol/kg (P < .01). Abscess uptake was significantly reduced (P < .01), probably as a result of the accelerated blood clearance. To investigate whether this lipid dose effect is species-specific, the biodistribution of PEG liposomes was also studied at various lipid dose levels in rabbits. Four groups of five rabbits each received 99mTc-PEG liposomes at either 1.0, 0.5, 0.1, or 0.02 μmol of phospholipid/kg b.wt. The biodistribution as determined ex vivo (i.e., counting the radioactivity in the tissues after dissection) was studied at 4 h p.i. Results are shown in Fig. 1. Compared with the data obtained at the highest dose level of 1.0 μmol/kg, significant differences were observed at the lowest dose level of 0.02 μmol/kg. The blood level at 4 h p.i. was reduced by 50% (P < .01). As observed in the studies in rats, abscess uptake was reduced at the two lowest dose levels. At the 0.02 μmol/kg dose level, radioactivity in the liver and spleen was strongly elevated (P < .05), whereas uptake in all other organs was markedly decreased.

In addition, the blood clearance of the radiolabeled liposomes was studied in rabbits by drawing blood samples at several time points p.i. As depicted in Fig. 2, removal of the PEG liposomes from the blood was relatively slow at the highest lipid dose levels (1.0 and 0.5 μmol/kg; τ1/2α = 10.4 and 9.8 h, respectively), which is concordant with our observations in previous studies. At the 0.1 μmol/kg dose level, the initial clearance was faster (τ1/2α = 6.7 h), whereas at the lowest dose level of 0.02 μmol/kg, the circulatory half-life appeared even more reduced (τ1/2α = 3.5 h). The main differences in the clearance profiles were observed in the distribution phase (τ1/2α), whereas the clearance pattern during the elimination-phase (τ1/2β) remained similar at all lipid dose levels.

**TABLE 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Suspected Focus</th>
<th>Phospholipid</th>
<th>Scintigraphy</th>
<th>Follow-up</th>
<th>Procedure</th>
<th>Result</th>
<th>τ1/2α</th>
<th>τ1/2β</th>
<th>t i/min</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>64</td>
<td>Hip</td>
<td>0.5</td>
<td>+</td>
<td>Ig. Bo</td>
<td>Bursitis</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>56</td>
<td>Abdomen</td>
<td>0.5</td>
<td>+</td>
<td>Ba</td>
<td>M. Crohn</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>57</td>
<td>Hip</td>
<td>0.1</td>
<td>–</td>
<td>Ig</td>
<td>No infection</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>45</td>
<td>Spine</td>
<td>0.1</td>
<td>–</td>
<td>Ig</td>
<td>No infection</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ig, 111In-IgG scan; Bo, Bone scan (99mTc-MDP); Ba, Barium enema; +, positive scan; –, negative scan.
of the radiolabel at various PEG liposome doses in rabbits are shown in Fig. 3. High blood levels, represented by a clearly visible heart region and large vessels, were observed on the scintigraphic images of infected rabbits that received lipid doses of 1.0, 0.5, and 0.1 $\mu$mol/kg. At these doses, liver, spleen, and kidneys were visualized. Abscess uptake was well visualized at the 1.0 and 0.5 $\mu$mol/kg dose level. At the 0.1 $\mu$mol/kg dose level, abscess uptake was less pronounced; at 0.02 $\mu$mol/kg, the lowest dose level, the abscess was not visible, coinciding with rapid blood clearance. At 4 h p.i., the blood pool activity was very low. Activity mainly shifted to the liver and, to a lesser extent, the spleen. With decreasing lipid dose, increased renal elimination of the radiolabel was observed, as shown by the well-visualized bladder. In addition, bone marrow uptake was markedly higher at the lowest lipid dose. Elevated uptake in the liver and bone marrow was visible at 30 min and afterward.

To investigate whether saturation of the mononuclear phagocyte system (MPS) or any other liposome consumptive system in the body played a role in this phenomenon of rapid clearance, two groups of rabbits received a first injection with either 0.5 ml of PBS (control) or 0.5 ml of 0.1 $\mu$mol/kg PEG liposomes. One hour after the first injection, rabbits received 0.02 $\mu$mol/kg $^{99m}$Tc-PEG liposomes; images were recorded 1 h after this injection (data not shown). The rabbits of the control group (injected with PBS) showed enhanced removal of the radiolabeled liposomes from the blood pool, in line with the results obtained at the 0.02 $\mu$mol/kg dose level discussed above. After injection of the second dose (0.02 $\mu$mol/kg), the rabbits of the other group (injected twice with liposomes) showed a distribution similar to that observed at higher doses, indicating saturation of the MPS or any other system involved in the removal of liposomes from the blood during the first PEG liposome injection.

To study the role of macrophages on the biodistribution of PEG liposomes at a low dose level (0.02 $\mu$mol/kg), macrophages in rabbits were depleted by a single injection of large multilamellar liposomes containing clodronate. Control rabbits received liposomes of the same lipid composition containing PBS instead of clodronate. Acid phosphatase staining of sections of liver and spleen from rabbits treated with clodronate liposomes confirmed the almost complete elimination of macrophages from these tissues. The macrophage-depleted rabbits showed a normal biodistribution, comparable with rabbits injected with higher doses of PEG liposomes. Both of these groups showed high blood levels (0.50 ± 0.06%ID/g) and significantly lower liver uptake (0.19 ± 0.07%ID/g). Control rabbits (not depleted from macrophages) showed a biodistribution pattern similar to the biodistribution observed after a single injection of low-dose PEG liposomes and elevated liver uptake. Accelerated blood clearance was observed (0.35 ± 0.05%ID/g, 4 h p.i.), as well as elevated uptake in the liver (0.49 ± 0.04%ID/g, 4 h p.i.). These data strongly indicate that macrophages are involved in the rapid clearance of low lipid dose PEG liposomes from the circulation.

To investigate whether the dose effect as observed in rats and rabbits could also be observed in humans, the blood clearance and biodistribution of radiolabeled PEG liposomes at two lipid dose levels was studied in patients. As part of an ongoing clinical trial, we investigated two patients at a lipid dose level of 0.5 $\mu$mol/kg (the standard dose used in that study) and two patients at a dose level of 0.1 $\mu$mol/kg. The clinical characteristics of the patients, the injected phospholipid dose, the results of the $^{99m}$Tc-PEG liposome scan, and

![Fig. 2. Blood clearance of $^{99m}$Tc-PEG liposomes at four phospholipid dose levels determined in rabbits with an E. coli infection in the left thigh muscle. Values are expressed as %ID/g of blood. The error bars represent S.D. (n = 5/group). ○, 0.02 $\mu$mol/kg; ○, 0.1 $\mu$mol/kg; ●, 0.5 $\mu$mol/kg; ■, 1.0 $\mu$mol/kg.](image)

![Fig. 3. Scintigraphic images of rabbits with an E. coli abscess in the left thigh muscle 4 h after injection of $^{99m}$Tc-PEG liposomes at four phospholipid dose levels.](image)
the pharmacokinetic data are summarized in Table 1. Whole body scintigrams of patient 2 (high dose) and patient 4 (low dose) are shown in Fig. 4. Patients 1 and 2, injected with liposomes at the highest dose level, both showed a normal biodistribution with activity in the heart region and the large vessels and uptake in the liver and the spleen. In patient 2, suffering from inflammatory bowel disease, focal uptake in an affected bowel segment was visible. This disease entity was confirmed by barium enema. By drawing regions of interest over the liver and the spleen, %ID in both organs was calculated at 4 h p.i. In patients 1 and 2, liver uptake was 9 and 14%ID, respectively, whereas splenic uptake was 1 and 3%ID, respectively.

Patients 3 and 4, injected with the lower lipid dose (0.1 μmol/kg), showed strongly increased activity in liver, spleen, and bone marrow and decreased activity in the heart region, indicating enhanced clearance of the 99mTc-PEG liposomes from the circulation. Uptake in the liver 4 h p.i. for patients 3 and 4 was 30 and 23%ID, respectively, which is approximately 2.5 times higher than at the 0.5 μmol/kg lipid dose level. Splenic uptake in both patients was 7 and 17%ID, respectively, which is nearly 5-fold higher than observed at the 0.5 μmol/kg dose level. At 24 h p.i., uptake of the radiolabel in the intestines was noted in both patients, indicating hepatobiliary excretion of the radiolabel after processing of the liposomes in the liver.

**Discussion**

In this study we have shown that the lipid dose of PEG liposomes greatly affects the circulation time. At the low dose, the PEG liposomes were cleared rapidly from the circulation. The liposomes were mainly taken up by the MPS tissues (liver, spleen, and bone marrow). Several studies (Allen and Chonn, 1987; Gabizon and Papahadjopoulos, 1988; Woodle and Lasic, 1992) have reported that liposomes coated with PEG have long-circulating times independent of the administered lipid dose. Most of these studies focused on the suitability of PEG liposomes for drug delivery in therapeutic applications. In general, in these applications relatively high lipid doses were used. In contrast, in nuclear medicine applications it is common to administer tracer amounts of radiopharmaceuticals. Studies on the blood clearance of PEG liposomes have been carried out using doses ranging from 4 to 400 μmol of lipid/kg b.wt. (Allen and Hansen, 1991), whereas PEG liposomes used for infection imaging were injected at lipid dose levels of approximately 0.5 μmol/kg (Boerman et al., 1997; Dams et al. 1998, 1999, 2000). Recently, Utkhede and Tilcock (1998) were the first to report on accelerated blood clearance of PEG liposomes after administration of very low lipid doses in rabbits. They described strongly decreased circulatory half-lives at the 0.16 μmol/kg dose level. In agreement with their studies, we found enhanced clearance at the 0.02 μmol/kg dose level but normal circulatory half-lives at a lipid dose of 0.1 μmol/kg. This discordance may—at least partially—be related to differences in PEG liposome formulations. Utkhede and Tilcock (1998) coated liposomes with PEG with a molecular weight of 5000, whereas we used PEG-2000. Maruyama et al. (1992) have shown that PEG-5000 liposomes have significantly shorter circulation times and higher uptake in liver and spleen compared with PEG-2000 liposomes. This would point to more intensive opsonization of PEG-5000 liposomes and possibly a higher “threshold” dose above which the long circulation kinetics is lipid dose-independent. In line with our results, Utkhede and Tilcock (1998) also report on increased renal elimination of the radiolabel with decreasing lipid dose, indicating enhanced processing and degradation of the PEG liposomes within the phagocytic cells in the liver and the spleen.

More importantly, in this study the lipid dose phenomenon was also observed in humans. In humans, rapid elimination of the radiolabeled PEG liposomes from the circulation was observed at a phospholipid dose of approximately 0.1 μmol/kg. The biodistribution pattern was similar to that observed in the rats and rabbits, with high uptake in liver, spleen, and bone marrow. Most likely, the liposomes are taken up by cells of the MPS present in liver, spleen, and bone marrow. In addition, bowel uptake was noted in one patient, indicating processing of the liposomes trapped in the liver and subsequent hepatobiliary excretion.

At present, there is no consensus about the mechanism of elimination of PEG liposomes from the circulation by the MPS. Several reports suggested the presence of proteins that may act as opsonins for these liposomes. From our experiments, as well as from literature (Oja et al., 1996), it appears that this opsonin pool of proteins is limited in the case of PEG liposomes. Oja et al. (1996) hypothesized that the amount of protein bound to the liposomes dictates the liposome clearance properties. In this hypothesis, high doses of liposomes correspond with relatively low protein amounts per particle and therefore extend circulation times. This suggests that “saturation” is in fact the result of depletion of blood opsonins, thus not resulting from MPS saturation. In our experiments, with two subsequent injections of liposomes, the second (lower lipid dose) injection showed a normal biodistribution, suggesting that the pool of blood opsonins was depleted by the first (higher lipid dose) injection. Although the clearance mechanism is still not fully understood,

Conclusion

Our studies demonstrated that the biodistribution of PEG liposomes is lipid dose-dependent and dramatically altered at very low lipid doses. Therefore, the clinical use of 99mTc-PEG liposomes for imaging infection and inflammation requires administration of lipid doses of at least 0.5 μmol/kg b.wt.

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References


Send reprint requests to: Peter Laverman, University Medical Center Nijmegen, Department of Nuclear Medicine, P.O. Box 9101, NL-6500 HB Nijmegen, the Netherlands. E-mail: p.laverman@nugen.aan.nl