Suppression of liver uptake of liposomes by dextran sulfate 500

(drug delivery/liver blockade/tissue distribution/lipid vesicles)

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ABSTRACT The effect of dextran sulfate (DS, 500,000 M,) and multilamellar vesicles (MLV) as liver blockade agents has been investigated in mice. Intravenous injection of unlabeled MLV prior to radioactive MLV caused moderate reduction in the liver uptake and increased tibia, lung, and spleen uptake. More drastic differences were observed with intraperitoneal injection of DS. When tested in the range of 0–50 mg of DS per kg of body weight, maximal liver blockade occurred at a dose of 50 mg. By using 50 mg of DS per kg, maximal liver blockade occurred at 12 hr after DS injection. The liver blockade was temporary, ending within 48 hr. The intraperitoneal route of injection for DS was more effective for liver blockade than the intravenous route.

Since Bangham et al. (1) originally prepared phospholipid vesicles as model membranes, many investigators have recognized their potential role as carriers of biologically active materials, such as enzymes (2), chelators (3, 4), viral nucleic acids (5), antitumor drugs (6–11), antibiotics (12–14), and immunogens (15–18), and hormones (19). However, rapid liver uptake of intravenously (i.v.) injected liposomes is a major obstacle to clinical use of liposomes, because it does not allow the liposomes to reach other important organs.

Approaches to overcome this problem have been reported by several laboratories: tumor-specific antibodies have been bound to the surface of liposomes to direct them selectively toward tumor cells in vivo (20), small liposomes have been shown to stay in circulation longer (21), and temperature- and pH-sensitive liposomes have been prepared that deliver their contents to specific areas of the body (22, 23).

Alteration in the surface composition of liposomes has also been used for selective delivery: liposomes with galactose-containing surfaces are selectively delivered to the liver (24) and, in particular, to the hepatocytes of the liver (11, 25, 26), liposomes with membrane insertion of either phosphatidylserine (27, 28) or aminomannose are targeted selectively to macrophages (29), and liposomes that include sialogangliosides in the membrane have somewhat reduced liver uptake (24).

Many of these approaches have improved the delivery of liposomes to specific organs but with little effect on the removal of a large fraction of the circulating liposomes by the liver. There have been some attempts to reduce liver uptake by saturating the reticuloendothelial system (RES) by pretreatment with a high dose of multilamellar vesicles (MLV), resulting in a transient decrease of hepatic uptake (30). It has also been shown that pretreatment with reversed-phase evaporation vesicles (REV) decreases liver uptake of a second dose of similar vesicles (31). Pretreatment with either REV or small unilamellar vesicles (SUV) induces reticuloendothelial blockade, resulting in slower blood clearance of liposomes (32). Pretreatment with latex beads also increases the time that REV remain in the circulation, but this approach does not have a significant effect on uptake of vesicles by the liver (32). Proffitt et al. (33) have reported that aminomannose-modified SUV cause RES blockade in tumor-bearing mice and higher tumor uptake of SUV but only modest reduction in the liver uptake.

Compounds that are toxic to liver macrophages have also been used to block the liver uptake of liposomes. Tanaka et al. (34) have used methyl palmitate, which is toxic to macrophages, and Souhami et al. (35) have used dextran sulfate M, 500,000 (DS), known to be toxic to hepatic macrophages (36), for reticuloendothelial blockade to alter tissue distribution of liposomes.

In this paper we report further studies of the use of DS to reduce liver uptake of multilamellar liposomes. We have investigated: (i) the effect of various doses of intraperitoneally (i.p.) injected DS on the tissue distribution of MLV; (ii) the length of time of the effect of i.p. injected DS on the liver distribution of MLV; (iii) the relative effectiveness of i.p. vs. i.v. injection of DS; (iv) DS liver blockade in comparison with nontoxic MLV liver blockade; and (v) the effect of a combination of two different liver blockade agents.

MATERIALS AND METHODS

L-α-Distearoyl phosphatidylcholine, stearylamine, cholesterol, and DS sodium salt with M, 500,000 were obtained from Sigma. Deferoxamine mesylate (DF; Desferal) was purchased from CIBA Pharmaceutical. Amersham/Searle was the source of radioactive iron in the form of 59FeCl3 in 0.1 M HCl (1 mCi/ml; 1 Ci = 3.7 × 1010 Bq). The mice used were 3–5-month-old female Swiss Webster obtained from Simonsen Laboratories (Gilroy, CA).

Preparation of Liposomes. Liposomes containing, in the aqueous compartment, DF and a trace amount of 59Fe-labeled DF (59Fe-DF) were prepared according to methods described by Guilmette et al. (37). Distearoyl phosphatidylcholine, cholesterol, and stearylamine in the molar ratio of 1.5:1.0:0.40 were dried in a round-bottom flask. An aqueous phase containing 75 mg of 59Fe-DF per ml of water was typically added to 38 mg of total lipids in the flask and stirred at 60°C for 10 min. Unencapsulated 59Fe-DF was removed by three successive centrifugations at 3,015 × g for 10 min, each time resuspending the pellet in 5–7 ml of phosphate-buffered saline (P/NaCl).

In Vivo Liver Blockade. DS in P/NaCl was injected either i.p. or i.v., in dosages ranging from 10 to 50 mg/kg of body weight. In the controls, only P/NaCl was injected instead of DS. Each group included four mice. For an experiment in which a combination of liver-blocking agents was used, 50 mg of DS was administered.

Abbreviations: DS, dextran sulfate M, 500,000; MLV, multilamellar vesicle(s); SUV, small unilamellar vesicle(s); REV, reversed-phase evaporation vesicle(s); i.v., intravenous(ly); i.p., intraperitoneal(ly); RES, reticuloendothelial system; DF, deferoxamine; % ID, percent injected dose.

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per kg was injected by the i.p. route. After waiting 22 hr MLV were then injected, 2 mg of lipid mixtures per mouse by the i.v. route, followed by MLV with encapsulated 59Fe-DF by the i.v. route at 24 hr.

Measurement of Tissue Uptake of Radioactivity. When the mice were sacrificed, they were weighed and "% of injected dose" (% ID) in various tissues was determined from the total radioactivity injected into each mouse. Then the % ID per g in each tissue was calculated with the assumption that blood comprises 7.3% of the total body weight of animals (38). Blood was collected from the jugular vein and weighed, the radioactivity was counted, and dose per g was calculated. The activity of each tissue sample was determined with a Beckman Biogamma counter.

RESULTS

Uptake of MLV as a Function of Dosage of DS. The effects of increasing doses of DS as a liver-blocking agent are shown in Fig. 1. In all uptake experiments shown in this figure, the DS was given i.p., followed 24 hr later by an i.v. injection of MLV encapsulating radioactive deferoxamine (59Fe-DF). Two hours later the tissues shown were taken for analysis of radioactivity. As the DS dosage increased from 0 to 50 mg/kg of body weight, spleen, lung, and tibia showed a large increase, whereas liver showed a concomitant decrease. Other tissues (heart, small intestine, kidney, brain, stomach, and large intestine) omitted from the graph were measured only at the end points of the DS concentration range and showed very small uptake compared to lung, spleen, tibia, and liver. Fifty milligrams of DS per kg appears to be about the maximal dose that can be tolerated without significant lethality (39).

A full time-course experiment was conducted with 50 mg of DS per kg of body weight given i.p., followed at a specified interval by an i.v. injection of 59Fe-DF containing MLV, followed 2 hr later by tissue analysis.

As shown in Fig. 2, the blockade effect peaked at different times in different tissues. The full effect of the blockade appeared in spleen, tibia, and liver in 6–12 hr and in lung in 24 hr and was negligible in blood. In all of the tissues that did show
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FIG. 3. Effect of DS injection, 50 mg/kg of body weight, either by i.p. or i.v. route on the uptake of MLV (DS injected at hour 0; MLV injected i.v. at hour 24; sample taken at hour 26). Each value represents the mean ± SEM of four mice.

FIG. 4. Uptake of MLV as a function of time between MLV injection and tissue sampling (DS or Pr/NaCl injected i.p. at hour 0; MLV injected i.v. at hour 24; sample taken at hour 25, 27, or 30). Each value represents the mean ± SEM of four mice.

an effect, the tissue activities were back to preblockade levels by 48 hr after the DS injection.

The effect 24 hr after injection of i.v. DS on MLV uptake is shown in Fig. 3. Spleen, tibia, and blood uptake of MLV in mice that had received DS by the i.v. route was similar to uptake in mice that had received DS by the i.p. route. The effect of DS injected by the i.v. route was lower lung uptake and higher liver uptake of MLV compared to DS injected by the i.p. route.

Uptake of MLV as a Function of Time. Experiments were then conducted with 50 mg of DS per kg of body weight given i.p. (or omitted), followed by 59Fe-DF containing MLV in 24 hr, followed by tissue analysis of radioactivity in 1, 3, or 6 hr. In the absence of DS injection the tissue distribution is quite stable over the sampling range of 1–6 hr after MLV injection, as shown in Fig. 4. In the presence of the DS, the tissue distribution over that sampling range is quite stable in the tibia, liver, and blood, but it appears to increase gradually in spleen and decrease gradually in lung. Actual MLV uptake values for spleen, lung, and tibia with DS injection were very high, at all of the time points tested, compared to tissues in the absence of DS injection. The liver uptake of MLV in the DS-injected mice was very low compared to liver in the absence of DS. Blood circulation of MLV after DS injection increased 9, 4, and 3 times at 1, 3, and 6 hr, respectively.

Uptake of MLV as a Function of Blockade Agent. In the previous experiments, DS was used as the blocking agent. Another blocking technique is to saturate the liver with MLV before administration of additional MLV expected to reach the other tissues. As shown in Fig. 3, i.v. injection of MLV (2 mg of lipids per mouse) caused only moderate reduction in the liver uptake with moderate increase in lung and tibia and a very large increase in spleen uptake. However, i.p. injection of DS (50 mg/kg of body weight) was more effective in liver blockade, resulting in very high lung, spleen, and tibia uptake. When both MLV (2 mg of lipids per mouse) and DS (50 mg/kg of body weight) were used in combination as blocking agents, no further improvement in the liver blockade was observed.

DISCUSSION

We have tested the effect of DS, which has been reported to be toxic to hepatic macrophages (36, 40), on the tissue uptake and distribution of positively charged MLV. As the dose of DS increased to 50 mg/kg of body weight, MLV uptake by liver decreased. This reduction in the liver uptake of MLV made liposomes available to other organs, such as tibia (bone marrow), lung, and spleen for higher uptake.

Because of the toxic nature of DS, we studied the length of the suppression in the liver uptake. Our results show that the uptake of MLV by liver drops as early as 2 hr after DS injection. Liver suppression due to DS reached a maximum 12 hr after DS injection. At 24 hr after DS injection, liver uptake was 70% lower than control mice but had started to recover. Forty-eight hours after DS injection, liver uptake was similar to that in controls, suggesting either the recovery of liver macrophages from the DS toxicity or the replacement of damaged macrophages by new cells.

Our results are in agreement with Souhami et al. (35), who have shown that i.v. injection of 0.75 mg of DS per 20- to 30-

mouse (30 mg/kg) 2 hr prior to neutral MLV reduced the liver uptake by 35% at 30 min after injection. Their liver blockade caused 95% increase in spleen and 36% increase in lung uptake of MLV. Our results demonstrate much higher effects than Souhami et al. (35) reported on both the liver suppression as well as increased uptake in bone marrow, spleen, and lung.

Even though DS is reported to be toxic to hepatic macro-
phages, our results suggest that such effects may be only temporary, lasting <48 hr after injection. If the effects of DS are tolerable, which seems to be the case in mice, it might be possible to improve significantly the therapy of lung tumor, lymphoma, or leukemia by encapsulating antitumor drugs in liposomes and injecting them after DS blockade. A second application of liver suppression would be in the detection of metastases in either lung or spleen by using vesicle-encapsulated radioactive agents. Because of high liver uptake, the detection of tumor or metastases in either lung or spleen by liposome-encapsulated radioactive agents is very difficult. Radioactivity taken up by liver increases the difficulty of imaging either lung or spleen. This problem might be resolved by drastic reduction in the liver uptake of liposomes with the use of DS.

Our results also show that the RES can be blocked to a certain extent by i.v. injection of MLV. Injection of unlabeled MLV prior to injection of radioactive MLV resulted in a 23% reduction in the liver uptake of the labeled liposomes. This moderate drop in the liver uptake was accompanied by higher uptake in tibia (bone marrow), lung, and spleen.

Our results are consistent with the work of Ellens et al. (31), who have shown that i.v. injection of unlabeled REV 1 hr prior to injection of radioactive REV reduced the liver uptake by 50% and spleen uptake by 21%. Kao and Juliano (32) have used latex beads as an agent to block the RES. After a 1-hr blockade with latex beads, they injected REV by the i.v. route and followed the tissue distribution 2 hr later. This approach had no effect on liver uptake, but spleen uptake was decreased by 45%, and substantially higher lung uptake was observed.

The amount of MLV circulating in the blood after DS injection was about 9, 4, and 3 times higher at 1, 3, and 6 hr, respectively. Ellens et al. (31) have observed an increase of 5 times in the blood level of REV when the RES is first blocked with REV. Abra et al. (30) observed a 29 times higher blood level of MLV at 1 hr after MLV blockade. This level was sharply reduced after 6 hr. However, Abra et al. (30) filtered their MLV through a 1-mm pore-size Nucleopore membrane. The smaller MLV used in their experiments, compared to our unfiltered MLV, could have caused their observed higher blood levels.

Our results suggest that DS is more effective in liver blockade than MLV and might be useful as a liver blockade agent, allowing liposomes to accumulate in the spleen, lung, and bone marrow. This approach could provide a significant opportunity for tumor treatment as well as tumor diagnosis by radiolabel imaging techniques.

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