Suppression of Liver Uptake of Orally Fed Liposomes by Injection (I.P.) of Dextran Sulfate 500

Nirmalendu Das, Mukul Kumar Basu and Manoj Kumar Das

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Iodine labelled human immunoglobulin-G encapsulated liposomes were administered orally to rats. Distribution of radioactivity was checked in various tissues and in portal blood. The effect of dextran sulfate (DS 500,000 m. wt., liver blockade agent) injection (i.p.) on the liver uptake of liposomes and on the amount of liposomes appearing in the portal blood from the gastrointestinal tract have been studied. An increased amount of radioactivity was observed in the portal blood and the amount of radioactivity in the liver decreased appreciably after injection of dextran sulfate. In both the cases the action of dextran sulfate started 2 hours after injection and reached maximum at 12 hour, falling slightly at 24 hour.

INTRODUCTION

In recent years, there has been increased interest in the use of liposomes as a drug delivery system. Oral administration of drugs would be preferable not only for convenience but also because it enters the blood via the portal circulation. It is known that certain drugs and bioactive molecules when given orally could not reach the periphery intact. It was shown that intragastric administration of insulin liposomes led to hypoglycaemic activity in diabetic animals (1, 2). We reported earlier that bioactive molecules encapsulated in liposomes could be detected intact in the portal blood when given orally (3), but all the absorbed material in the portal circulation from the

Biomembrane Div., Indian Institute of Chemical Biology, Calcutta 700032, India.
1 Present address: Regional Medical Research Centre, Bhubaneswar 751005.
2 To whom correspondence should be addressed.

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gastrointestinal tract would be transported to the liver where the liposomes would be degraded. Alteration in the surface composition of liposomes had been tested by several groups for selective delivery. Sugar-coated liposomes when injected i.v. were selectively taken up by the liver (4, 5). Approaches have been improved for the delivery of liposomes to specific organs with little alteration of the liver uptake. Therefore, the removal of a large fraction of circulating liposomes takes place by the liver. Decreased liver uptake of liposomes was observed by saturating the reticuloendothelial system by pretreatment with a high dose of multilamellar vesicles (6). Pretreatment with small unilamellar vesicles also causes reticuloendothelial blockade resulting in slower blood clearance of liposomes. Compounds that are toxic to liver macrophages had also been used to block the liver uptake of liposomes. Several compounds, e.g. methyl palmitate (7) and dextran sulfate (8), have been tested for the reticuloendothelial blockade to alter the tissue distribution of liposomes. In this present report, we have demonstrated that, by blocking the liver with dextran sulfate, the liposomal uptake from portal blood by liver can be suppressed, and it may facilitate the transport of those vesicles to other tissues.

MATERIALS AND METHODS

Dipalmitoyl phosphatidyl choline (DPPC), cholesterol (Chol), and dextran sulfate sodium salt (DS) with m. wt. 500,000, human immunoglobulin G (HIgG) and dicetyl phosphate (DCP) were obtained from Sigma. Carrier-free Na$^{125}$I was from BARC, Bombay, India. Radioiodinated HIgG ($^{125}$I-HIgG) was prepared following the published method (9).

Preparation of Liposomes

Liposomes were made with a mixture of phospholipid/Chol/DCP in a 7:2:1 molar ratio according to the method of Gregoriadis and Ryman (10). In short, a thin dry film consisting of lipid mixture (25 mg of lipids) was dispersed in 0.025 M phosphate-buffered saline 0.15 M NaCl, pH 7.2 (PBS) containing 5 mg/ml HIgG mixed with a trace amount of $^{125}$I-HIgG. The dispersion was completed by brief sonication (30S) with a MSE ultrasonicator. The liposomes with entrapped protein were separated from the unencapsulated materials by repeated washing in buffer by ultracentrifugation at 105,000 $g$ for 60 min.

Animal Experiments

Male Swiss albino rats (IICB strain) weighing approx. 100–110 g were used. Oral administration of the test material was given to the rats 30 to 40 min after they were being fed a standard diet. DS in buffer (PBS) was injected i.p. in dosage ranging from 10 to 50 mg/kg of body weight. In controls, PBS was injected i.p. in place of DS. Liposomal suspension 0.5 ml (1.0–1.5 mg lipids) containing $13-15 \times 10^5$ cpm $^{125}$I-HIgG was introduced intragastrically through a polythene tubing into each animal. As a control, free $^{125}$I-HIgG ($13-15 \times 10^5$ cpm) was also fed. After appropriate
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Time intervals blood was collected from portal vein under ether anesthesia. The plasma was prepared by centrifugation at 2000 rpm for 15 min.

An identical dose of liposomes was introduced orally to different groups of rats (DS treated and untreated), and the animals were sacrificed at different times. The livers and spleens were removed and then washed with 0.9% NaCl solution and blotted with filter paper. The whole liver was digested in 30% KOH solution. The digested tissues (1.0 ml) and blood plasma (0.5 ml) were taken for radioactive counting.

RESULTS

Amount of Radioactivity in Different Tissues as a Function of Dosage of Dextran Sulfate

As presented in Fig. 1 the effect of increasing concentrations of DS on the amount of radioactivity in the liver, spleen and in blood from portal vein has been studied. DS was injected (i.p.) (0-50 mg/kg b.wt.) to rats and, 12 hours after injection, 125I-HIgG encapsulated liposomes were given orally. The radioactivity was examined in liver, spleen and portal blood one and a half hours after oral feeding. The level of radioactivity increased proportionally in spleen and portal blood, but decreased sharply in liver. The effects of DS injection (i.p.) on different tissues were optimal at a concentration of 40 mg/kg b.wt.

![Fig. 1. Effect of different dosage of DS injection (i.p.) on the amount of radioactivity in the liver ——; spleen ——— and in 0.5 ml portal blood ———, of rats fed with 125I-HIgG encapsulated DPPC liposome. DS injected (i.p.) at hour 0 and 13-15 × 10^5 cpm 125I-HIgG encapsulated DPPC liposomes were administered orally at hour 12; radioactivity was measured in different organs at hour 13.5. Each value represents the mean of 6 rats.](image)

Figure 2 depicts the pattern of radioactivity in liver, spleen and portal blood after an interperitoneal (i.p.) injection of dextran sulfate. Investigation was carried out with 40 mg of DS per kg of b.wt. given i.p. At different times after DS treatment, 125I-HIgG encapsulated liposomes were administered orally to rats and the distribution of radioactivity in 0.5 ml portal blood and in different organs was measured after 1.5 hours. In liver the level of radioactivity decreased with time of DS injection (i.p.), and the value reached maximum at 12 hours.
Fig. 2. Amount of radioactivity in liver ——, spleen —— as a function of time between injection (i.p.) of DS and oral feeding of $^{125}$I-HIgG encapsulated DPPC liposomes. DS injected (i.p.) 40 mg/kg b.wt at hour 0; $^{125}$I-HIgG encapsulated liposomes given orally to rats at 2, 4, 8 hour, etc., radioactivity measured 1.5 hours later.

**Presence of Liposomes in Portal Blood**

The blood from the portal vein of rats (DS treated and untreated) was collected after 1.5 hour of feeding $^{125}$I-HIgG encapsulated negatively charged DPPC liposomes. An increased amount of radioactivity was shown in the portal blood in the case of DS treated rats compared to control rats. The plasma was passed through a Sepharose 2B column to establish the presence of liposomes or undegraded HIgG in the portal blood plasma. In both the cases (Fig. 3a and 3b) about 50% of the radioactivity in the portal blood plasma was associated with liposomal peak. However the presence of intact protein seems to be little higher in DS treated rats compared to control animals.

**DISCUSSION**

In a previous report we demonstrated the appearance of intact liposomes and protein in portal blood following oral administration of liposome encapsulated protein to rats, and we concluded that some amount of liposomes could be absorbed into the portal blood from the gastrointestinal tract and portal blood streams would carry those liposomes and protein into the liver which disrupted the liposomes and protein. The absence of liposomes and protein in the cardiac blood supported that view (3).

In the present report, we examined the effect of DS on the amount of radioactivity in the liver, spleen and portal vein blood. After DS injection (i.p.), the liver uptake decreased 4 times whereas, by applying the same dose of DS, the uptake increased by 75% and 60% respectively in the spleen and portal blood (Fig. 2). Patel et al. (11) showed that i.p. injection of (50 mg/kg) DS in mice 12 hours prior to i.v. injection of...
multilamellar vesicles, suppressed the liver uptake 5 times at 2 hr, in comparison to the value obtained in absence of DS injection (i.p.). Our results, which support the work of Patel et al. (11), indicate that higher amounts of radioactivity, of which most is associated with the liposomes and the protein in the portal blood plasma, may be due to the weak clearance of those molecules from the portal blood because of the liver presaturated with DS. It is not known whether DS injection (i.p.) has any effect on the absorption of high molecular wt. compounds from the gastrointestinal tract.

It is known that DS is toxic to hepatic macrophages. Patel et al. (11) suggested that such an effect of DS might be temporary, and they concluded that after 48 hr liver macrophages would neutralize the DS toxicity or newly formed hepatic cells would synthesize new macrophages. The decrease of DS after 12 hr (Fig. 2) is in agreement with these views. It is suggested that by blocking the liver with DS, liposomes in portal blood may be directed to other tissues.

REFERENCES


