CHAPTER IV

SUPPRESSION OF LIVER UPTAKE OF ORALLY FED LIPOSOMES BY INJECTION (I.P) OF DEXTRAN SULFATE 500

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IV.1 Summary

^{125}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 100,000 m. wt.) injection (i.p.) on the liver uptake and on the amount of liposomal appearance in the portal blood have been studied. Increased amount of radioactivity was observed in the portal blood and the amount of radioactivity in the liver decreased appreciably by the injection of dextran sulfate. In both the cases the action of dextran sulfate started from 2 hour of injection and reached maximum at 12 hour and fell off slightly at 24 hour.

IV.2 Introduction

In recent years, there has been increased interest in the use of liposomes as 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 was shown that intragastric administration of insulin liposomes led to hypoglycaemic activity in diabetic animals (1-2). It was reported earlier that bioactive molecule encapsulated in liposomes could be detected intact

in the portal blood when it was given orally (3). But the absorbed material in the portal circulation from the 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 organ with little alteration of the liver uptake. Thus 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 unioamellar vesicles also causes reticuloendothelial blockade resulting to slower blood clearence 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 Chapter, it has been demonstrated that by blocking the liver with dextran sulfate the liposomal uptake by liver can be suppressed and it may facilitate the transport of those vesicles to other tissues.

IV.3 Materials and Methods

IV.3.1 Reagents

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.

IV.3.2 Methods

IV.3.2.1 Iodination of HIgG

Radioiodinated HIgG (^{125}I-HIgG) was prepared following the published method (9) described in Chapter II.

IV.3.2.2 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). This is performed as described in Chapter II.

IV.3.2.3 Animal experiments

Male swiss albino rats (IICB strain) weighing approx 100-110 g were used. Liposomes were administered orally to 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, only PBS was injected (i.p.) instead of DS.

Liposomal suspension 0.5 ml (1.0-1.5 mg lipids) containing 13-15 x 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 x 10^5 cpm) was also fed. After appropriate time intervals blood was collected from portal vein under ether anesthesia. The plasma was prepared by centrifugation at 2000 rpm for 15 min.

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.

IV.4 Results

IV.4.1 Amount of radioactivity in different tissues as a function of dosage of dextran sulfate.

As presented in Fig.1 the effect of increasing concentration 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 after 12 hour of injection, ^{125}I-HIgG encapsulated liposomes were given orally. The radioactivity was examined in liver,

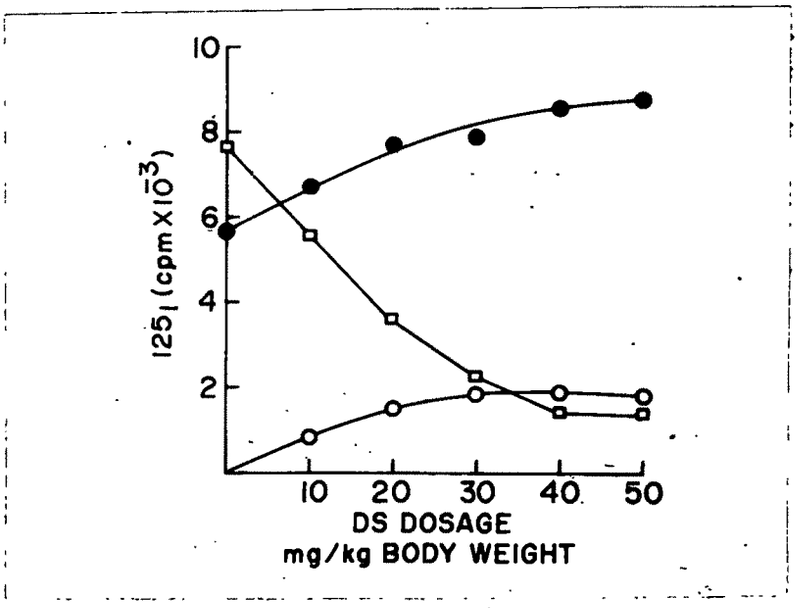

Fig.1 - Effect of different dosage of DS injection (i.p.) on the amount of radioactivity in the liver -□- ; spleen -O- and in 0.5 ml portal blood -●-, of rats fed with ^{125}I-HIgG encapsulated DPPC liposome. DS injected (i.p.) at hour 0 and 13-15 x 10^5 Cpm ^{125}I-HIgG encapsulated DPPC liposomes were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

spleen and portal blood after an hour and half the 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 the concentration of 40 mg/kg b.wt.

IV.4.2 Distribution of radioactivity in various tissues by the injection (i.p.) of Dextran Sulfate 500 at different times

Fig.2 depicts the pattern of radioactivity in liver, spleen and portal blood after the intraperitoneal (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 time after DS treatment, ^{125}I-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 of feeding. In liver the level of radioactivity decreased with time of DS injection (i.p.) but in portal blood the amount increased. The optimum effect of dextran sulfate is noticed at 12 h of treatment.

IV.4.3 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. Increased amount of radicactivity was appeared in the portal blood in case of DS treated rats compared to control rats.

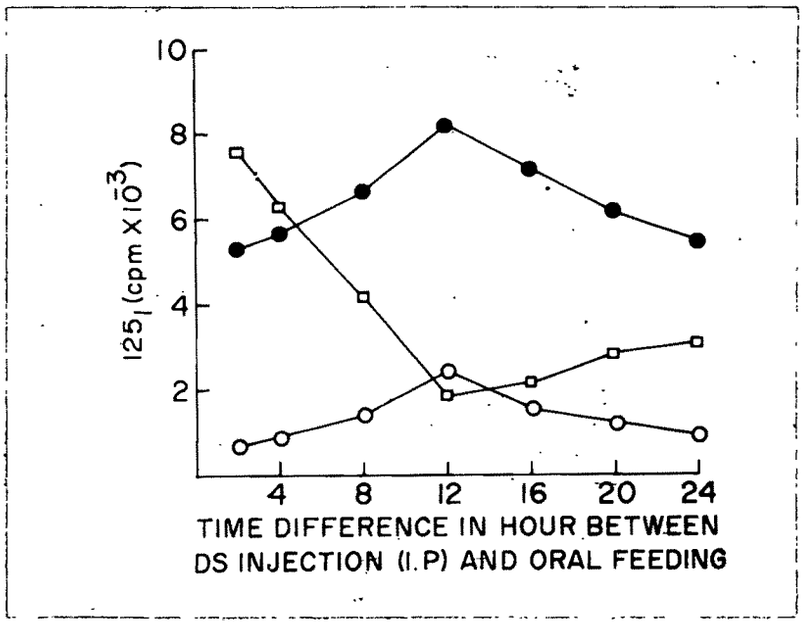

Fig.2 - Amount of radioactivity in liver -□-, spleen -O- and in 0.5 ml blood -●- as a function of time between injection (i.p.) of DS and oral feeding of ^{125}I-HIgG encapsulated DPPC liposomes.
DS injected (i.p.) 40 mg/kg b. wt at hour 0; ^{125}I-HIgG encapsulated liposomes given orally to rats at hour 2, 4, 8 etc., radioactivity measured at 1.5 hour of feeding.

The plasma was passed through a Sepharose 2B column to establish the presence of liposomes or undergraded 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. On the other hand the presence of intact protein seem to be little higher in DS treated rats compared to control animals.

IV.5 Discussion

In the previous Chapter the appearance of the intact liposomes and protein was detected in the portal blood following the oral administration of liposome encapsulated protein to rats. It was 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 proteins into the liver which eventually would disrupt the liposomes and proteins. The absence of liposomes and protein in the cardiac blood supported that view (3).

In this chapter, the effect of DS was examined on the amount of radioactivity in the liver, spleen and in 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 in the portal blood(Fig.2). Patel et al. (11) showed that (i.p.) injection of (50 mg/kg) DS in mouse 12 hour prior to multilamellar vesicle injection (i.v.) suppressed the liver uptake 5 times at 2 hr in comparison to the

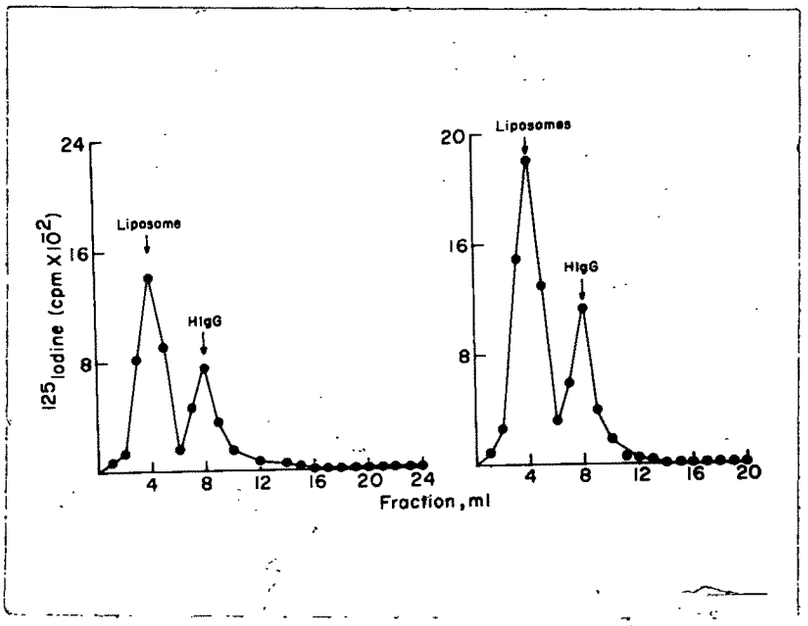

Fig.3 - Elution position of intact ^{125}I-HIgG and ^{125}I-HIgG encapsulated liposomes indicated with arrows. Elution profile from Sepharose 2B column (1x25cm) of 0.5 ml plasma taken from portal vein of rats 1.5 h of feeding ^{125}I-HIgG encapsulated DPPC liposomes.

a) Control experiment

b) DS treated (i.p.) at hour 0, ^{125}I-HIgG encapsulated liposomes were introduced orally at hr 12 and blood from portal vein taken 1.5 hr after the feeding. Radioactivity associated with 0.5 ml plasma were (a) 5500 cpm
(b) 5850 cpm

value obtained in absence of DS injection (i.p.). Present reports which support the work of Patel et al. (11) indicate that higher amount of radioactivity of which large part is associated with the liposomes in the portal blood plasma, may be due to the weak clearance of those molecules from the portal blood by the liver presaturated with DS. It is not known whether DS injection (i.p.) has any effect on the absorption of high molecular wt. compound from the gastro-intestinal tract.

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

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