CHAPTER III

EFFECT OF CHOLESTEROL CONTENT ON THE EFFICACY OF LIPOSOMES IN ORAL ADMINISTRATION

III.1 Summary

^{125}Iodine labelled human IgG encapsulated liposomes were prepared with different concentration of cholesterol. Cholesterol-rich and cholesterol-poor liposomes were made with the phospholipid and cholesterol by the molar ratio 7:2 and 1:1 respectively. The distribution of radioactivity of these cholesterol-rich and cholesterol-poor liposomes were checked in liver, and in blood from portal vein and heart. The time of retaintion of those vesicles in the intestine has also been studied. The radioactivity in the cholesterol-rich liposomes in retained in intestine for a higher period compared to that from cholesterol-poor. Higher amount of radioactivity was determined in liver in all the time upto 24 hours after feeding cholesterol-rich liposomes. Gel chromatography pattern of plasma from portal vein and heart indicated the presence of liposomes and protein. But the relative amount of liposomes and intact protein were dependent on both the phospholipid and cholesterol content of liposomes. In cholesterol-rich dipalmitoylphosphatidyl choline liposomes, protein was found upto 24 hr of feeding, in constrast the stability of protein encapsulated in cholesterol-poor liposomes decreased readily.

III.2 Introduction

The use of liposomes as potential carrier for oral delivery of drugs is under extensive investigation. Oral administration of insulin entrapped in liposomes was shown to reduce the blood glucose level of diabetic animals (1,2). Similar studies were conducted in other systems, namely with blood coagulation factor VIII in a hemophilic patient (3) and with enzyme uricase in chickens (4). In the previous chapter it was demonstrated that liposomes or intact protein were present in portal blood of rats following the oral administration of 6-CF and ^{125}I-labelled human immunoglobulin G encapsulated liposomes. Liposomes or protein were not, however, detected in cardiac blood. It was inferred that liver uptake of liposomes from portal vein disrupts the integrity of liposomes or protein prior to their appearance in cardiac blood (5). In the present study, liposomes with increased cholesterol concentration has been utilized for oral administration in order to maintain the integrity of those vesicles in the gastrointestinal tract. Several investigators have demonstrated the components of blood responsible for disruption of liposomes (6-8). The presence of free HIgG in portal blood could be due to destabilization of liposomes by plasma factors (5). Free labelled protein could be leaked from liposomes by the interaction of them with the plasma components in portal vein.

It is known that the structural stability of liposomes in biological milieu can be enhanced considerably by the

incorporation of high concentration of cholesterol in liposomal membranes (9,19). Cholesterol-rich liposomes remained considerably stable in blood of animals injected intravenously. The stability of cholesterol-rich liposomes was also studied when injected subcutaneously (11). High concentration of cholesterol in liposomes was suggested essential for the maintenance of their stability in the presence of various blood components (9) and thus the integrity of the liposomal membrane may be compromised by complement mediated damage and by the action of hydrophobic plasma protein, as well as by the action of phospholipases or phospholipid exchange protein (10).

III.3 Material and methods

III.3.1 Reagents : Reagents are same as described in Chapter II.

III.3.2 Preparation of liposomes

Liposomes were prepared with different concentration of cholesterol by following the method described in chapter II. Cholesterol-poor and cholesterol-rich liposomes were made with a mixture of phospholipid/cholesterol in a molar ratio of 7:2 and 1:1 respectively. In short, a thin dry film of lipids was suspended in 0.025 M PBS (0.15 M NaCl, pH 7.2) containing 5 mg/ml HIgG mixed with a trace amount of ^{125}I-HIgG. The dispersion was completed by brief sonication (30 S). The liposomes with entrapped protein were separated from the unencapsulated material by repeated washing in buffer by ultracentrifugation at 150,000 g for 60 min.

II.3.3 Radioiodination of human immunoglobulin G

Human immunoglobulin G was iodinated with $Na^{125}I$ by following the method described in Chapter II.

III.3.4 Animal experiments

Liposomal suspensions (0.5 ml, 1.0-1.5 mg lipids containing ^{125}I-HIgG 1.30-1.50 x 10^6 cpm) were introduced orally to Swiss Albino male rats (IICB strain, 100-110g b. wt.) 30/40 min after feeding a standard diet. Liposome was introduced into the stomach of each animal through a polythene tubing. As a control, free ^{125}I-HIgG of equivalent radioactivity was also fed. Blood was collected after appropriate time intervals in heparinized tubes from portal vein of rats under ether anesthesia. The plasma was prepared by centrifugation at 3000 rpm for 15 min. Similar doses of liposomes were fed to another group of rats and the animals were sacrificed at various times. The liver and intestine were removed, washed with 0.9% NaCl solution and blotted with filter paper. The whole tissue was digested in 30% KOH solution. The digested material (1.0 ml) was taken for radioactive counting.

III.3.6 Gel chromatography of blood plasma

Blood plasma from the portal vein was passed through Sepharose 2B column that was prestandardized with ^{125}I-HIgG entrapped liposomes. Similarly, cardiac blood was analyzed on a Sephadex G-100 column. The procedure was described

previously (5).

III.4 Results

III.4.1 Amount of radioactivity retained in small intestine of rats fed with different liposomes

The amount of radioactivity retained in the intestine was measured following the oral administration of ^{125}I--HIgG encapsulated cholesterol-rich PC and DPPC liposomes and the value is compared with the results obtained by using corresponding cholesterol-poor liposomes. The percentage radioactivity of the orally introduced dose in intestine (Fig.1) was higher in case of cholesterol-rich liposomes in comparison to the value obtained from cholesterol-poor. About 20% of the oral injected dose was found in intestine after 4 hr of feeding cholesterol-rich DPPC liposomes but less than 10% was observed in intestine at that time after the oral introduction of cholesterol-poor DPPC liposomes (Fig.1a). Similarly, the percentage of radioactivity in intestine was higher in case of cholesterol-rich PC liposomes compared to that obtained from cholesterol-poor. Between PC and DPPC liposomes of same cholesterol content the values were higher in case of latter.

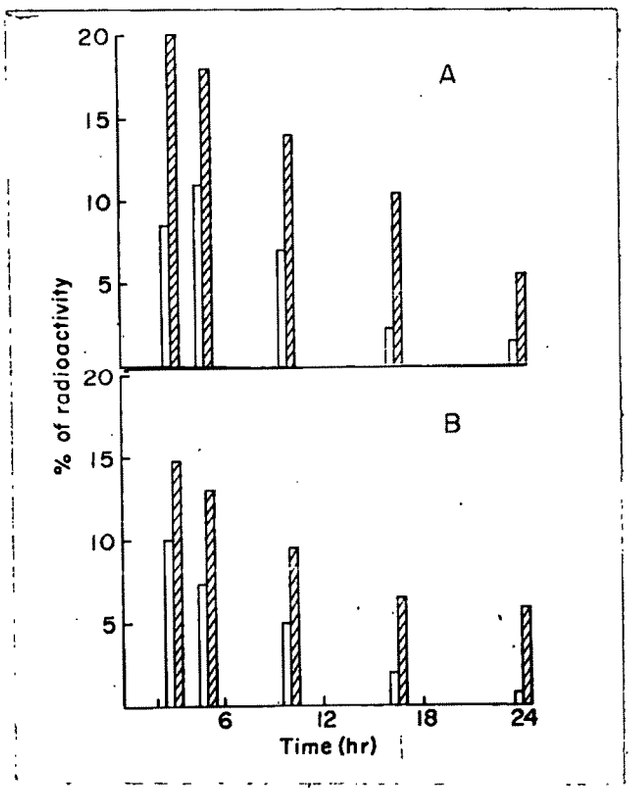

Fig.1 - Percentage of radioactivity in small intestine of rats fed with cholesterol-rich (▨) and cholesterol-poor (□) liposomes Fig.1a, DPPC-liposomes; 1b, PC-liposomes. At varying periods, intestines were removed, washed with 0.9% NaCl solution, blotted with filter paper and digested in 30% KOH solution. One ml of the digested materials was taken for radioactive counting

III.4.2 Distribution of radioactivity in the livers of rats fed with different liposomes

Amount of radioactivity in liver of rats fed with cholesterol-rich and cholesterol-poor DPPC liposomes was checked in different time of feeding of those vesicles (Fig.2a, 2b). The amount is higher in liver of rats given cholesterol-rich DPPC or PC liposomes in comparison to the value obtained from cholesterol-poor DPPC or PC liposomes. Liver uptake of liposomes is observed maximum at 5 hr of feeding DPPC or PC liposomes either of cholesterol-poor or cholesterol-rich. But much higher amount of radioactivity was detected in case of cholesterol-rich liposomes in comparison to cholesterol-poor vesicles. Appreciable amount of radioactivity was observed at that tissue even after 24 hr of feeding cholesterol-rich DPPC liposomes (Fig.2a). But the value fell off very sharply after 16 hr in case of PC liposomes with identical cholesterol concentration.

III.4.3 Presence of liposomes and protein in portal blood plasma

The percentage of radioactivity recovered with liposomes/protein decreased at a faster rate in cholesterol-poor than that in cholesterol-rich liposomes (Tables 1 and 2). At 3 hr of feeding PC liposomes, radioactivity was 15% in cholesterol-poor and 50% in cholesterol-high liposomes. Intact protein was detected even after 24 hr of feeding cholesterol-high DPPC liposomes. But no protein was detected after 8 hr of oral administration of cholesterol-poor

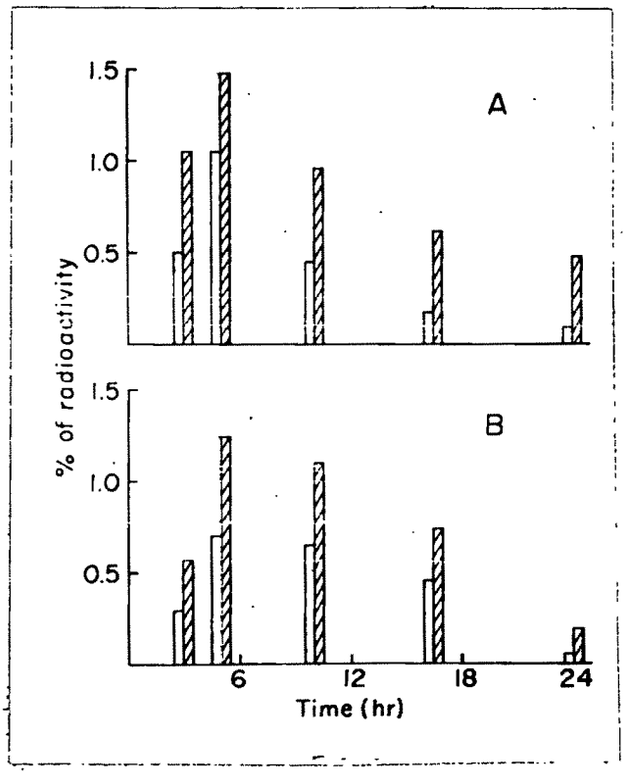

Fig.2 - Distribution of radioactivity in the liver of rats fed with cholesterol-rich (▨) and cholesterol-poor (□) liposomes Fig.2a, DPPC-liposomes, 2b, Pc-liposomes

TABLE I

Gel Chromatography (Sepharose-2B) of Plasma from Portal Vein of Rats Fed with [^{125}I]HIgG-Encapsulated DPPC Liposomes of Varying Cholesterol Concentrations.

[Results are expressed as percentage of total radioactive counts placed on the column (1x25 cm) prestandardised with liposomes and protein].

Liposomes	Composition	Time of taking blood	Radioactivity (%)	
			Liposome peak	Protein peak
DPPC	Cholesterol-rich	1.5	30	60
		3	50	45
		5	25	55
		8	8	30
		20	5	25
		24	-	10
	Cholesterol-poor	1.5	45	50
		3	25	50
		5	7	15
		8	-	-
		20	-	-

TABLE II

Gel Chromatography of Plasma from Portal Vein of Rats Fed with [^{125}I]HIgG-Encapsulated PC Liposomes.

[Conditions are same as described in Table I].

Liposomes	Composition	Time of taking blood	Radioactivity (%)	
			Liposome peak	Protein peak
PC	Cholesterol-rich	1.5	30	60
		3	50	35
		5	-	-
		8	-	-
	Cholesterol-poor	1.5	45	30
		3	15	20
		5	-	-

liposomes. In the initial period (5 hr), after feeding of cholesterol-high DPPC liposomes, almost all the radioactivity in portal blood were associated with either liposomes or protein peak.

III.5 Discussion

In this present chapter, liposomes with different cholesterol concentration has been used to study their efficacy in oral feeding programme. Gel chromatography of plasma from portal vein indicates that almost all the radioactive counts are associated with either liposomes or protein peak in the initial period (5 hr) after feeding of cholesterol-rich DPPC liposomes. It suggests negligible degradation of these liposomes in the gut.

Higher incorporation of cholesterol (50 mole %) in liposomes imparts greater stability to liposomally encapsulated protein. Intact protein and liposomes were detected in portal blood of rats fed with cholesterol-rich liposomes for a longer period than that in animals fed cholesterol-poor preparations. The majority of radioactivity recovered initially in portal blood was with liposomal/protein fractions during the early course of oral administration. It is evident that cholesterol-rich liposomes impart greater stability to encapsulated protein in the gut.

The majority of the count in portal blood remains with the protein fractions for rats fed with cholesterol-poor DPPC liposomes may be due to the destabilization of

those liposomes by plasma factors (12). The amount of radioactivity retained in the intestine is higher and for a longer period, as found with cholesterol-rich liposomes. It might suggest that the absorption of these liposomes is slower compared to cholesterol-poor liposomes. There are conflicting reports at present about the mode of absorption of liposomes from gut. Rowland and Woodley (13) using rat everted intestinal sac technique showed that absorption of intact liposomes could occur across mucosal cells. However, Patel et al. (14) using isolated perfused rabbit intestine found no evidence for the transport of intact liposomes across the intestine.

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