Role of lipoproteins in stability and clearance of liposomes administered to mice

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It is known that serum HD lipoproteins* can destabilize the lipid bilayer structure of liposomes by removal of phospholipid, so that the more destabilization, the more rapid the clearance of vesicles from the circulation of injected animals (Senior & Gregoriadis, 1982a,b; Gregoriadis, 1983). The aim of this work is to determine whether liposomal stability, as assessed by retention of entrapped markers, is improved in animals deficient in lipoproteins, and if so, whether this will lead to prolonged blood clearance. In addition we have investigated in vitro the effect of lipoproteins other than HD lipoproteins on liposomal stability, by using physiological concentrations of lipoproteins in the presence of lipoprotein-deficient plasma and at physiological temperature.

Induction of plasma lipoprotein deficiency in mice is brought about by administration of 4-amino-pyrazolopyrimidine, an adenine anti-metabolite (Senior et al., 1983). Doses of 60–70mg of drug/kg mouse wt. are given intraperitoneally on 3–4 consecutive days, leading to plasma cholesterol values of 0.2±0.1mmol/l (cf 3.4±1.3 mmol/l for normal animals). Small unilamellar liposomes composed of egg phosphatidylcholine with tracer [3H]phosphatidylcholine were prepared by sonication at 4°C with NaCl and 0.02% KCl (phosphate-buffered saline) and latent carboxyfluorescein and 3H radioactivity were measured as described by Senior (1974) and Mayhew (1983). The biodistribution of liposomes was followed by measurement of carboxyfluorescein-containing vesicles. Values from four mice in each group are percentages of the injected dose remaining in the blood after 30 min (see Fig. 1). However, animals given only one injection of the drug show a much slower rate of clearance of liposomes than do normal animals. Animals given three injections of the drug show an even slower clearance (1.24±0.10 half-life) (Fig. 1) approaching

* Abbreviation: HD lipoproteins, high density lipoproteins

Fig. 1. Clearance of latent carboxyfluorescein after injection of liposomes

Normal (○) or lipoprotein-deficient mice treated with three (■) or four (□) daily intraperitoneal injections of 4-amino-pyrazolopyrimidine were injected intravenously with carboxyfluorescein-containing phosphatidylcholine liposomes. Values from four mice in each group are percentages (± S.D.) of injected latent carboxyfluorescein per total mouse plasma (Senior et al., 1983).
that (2h) of similar cholesterol-rich liposomes (Senior & Gregoriadis, 1982b). When liposomes were injected intraperitoneally into normal animals, no latent carboxyfluorescein could be recovered in the circulation; however, when injected similarly into lipoprotein-deficient animals, latent dye was detected in the blood, with a peak of 10% of dose detected after 2h. Maintenance of liposomal stability in lipoprotein-deficient animals was also supported by studies in vitro, which indicate that [3H]phosphatidylycholine-labelled liposomes incubated with lipoprotein-deficient plasma at 37°C retain both entrapped carboxyfluorescein and [3H]phosphatidylycholine to a greater extent than do those incubated in normal plasma. This was shown by gel chromatography on Ultrogel AcA 34, which separates liposome-quenched carboxyfluorescein. Duplicate lop1 samples from in oitro, dose detected after 2 h. Maintenance of liposomal stability in lipoprotein-deficient animals is also supported by studies latent dye was detected in the blood, with a peak of when injected similarly into lipoprotein-deficient animals, this was shown by gel chromatography on Ultrogel AcA 34, which separates liposome-quenched carboxyfluorescein (Senior et al., 1983).

To determine whether other lipoproteins in addition to HD lipoprotein are active in destabilizing liposomes, lipoprotein-deficient plasma (0.5 ml) from 4-amino-phatidylcholine marker, and also entrapped and free chromatography on Ultrogel AcA 34, which separates liposome-quenched carboxyfluorescein. Duplicate lop1 samples from in oitro, dose detected after 2 h. Maintenance of liposomal stability in lipoprotein-deficient animals is also supported by studies latent dye was detected in the blood, with a peak of when injected similarly into lipoprotein-deficient animals, this was shown by gel chromatography on Ultrogel AcA 34, which separates liposome-quenched carboxyfluorescein (Senior et al., 1983).

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Antibody-directed liposomes: the development of a cell-specific cytotoxic agent

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Liposomes have often been proposed for use as drug carriers (Mayhew & Papahadjopoulos, 1983). The ability of liposomes to encapsulate drugs within their internal aqueous space or in the lipid bilayer makes them a versatile carrier system which may improve the efficacy of drugs in one of two ways. First, by acting as a slow release system, liposomes may modify the pharmacokinetics of drugs so as to prolong effective plasma drug levels. Second, being a microscopic carrier system, liposomes may transport and deliver drugs directly to cells.

The successful use of the second approach requires first that liposomes be directed specifically to the target cell, and second that they be capable of delivering the encapsulated drug to the cells once they reach them. Both of these characteristics can be examined by experiments in vitro, and we will here review our investigations which have been carried out to achieve these aims.

The targeting of liposomes to specific cell types is most readily achieved by attaching ligands to the liposome surface which will bind to determinants on the plasma membrane of the target cell. The most versatile ligands available are antibodies which can be produced to a wide variety of determinants on cells. In particular, monoclonal antibodies can be highly specific and preparations of monoclonals, unlike polyclonal antisera, contain very little antibody with no reactivity for the target antigen. We have developed several methods for the attachment of antibodies to liposomes. Our first procedure (Heath et al., 1981) involved the use of periodate-oxidized glycosphingolipids in the liposome membrane, which can be conjugated to antibodies by reductive amination with sodium cyanoborohydride. Subsequently, more efficient procedures were developed which involved thiol conjugation methods. In the first (Martin et al., 1981), phosphatidylethanolamine was modified by reaction with succinimidyl 3-(2-pyridydithio)propionate. The resultant modified lipid was incorporated into liposomes and reacted efficiently with rabbit Fab to form a dithiol bridge which can be reduced by glutathione or other thiol-reducing agents. A more stable linkage was achieved by a similar procedure (Martin & Papahadjopoulos, 1982) in which phosphatidylethanolamine was reacted with succinimidyl 4-(p-maleimidophenyl)butyrate to give a modified phospholipid with an alkylating moiety. This lipid, after incorporation into liposomes, reacted rapidly with rabbit Fab to form a thioether bond which cannot be reversed by reducing agents. We have also efficiently conjugated monoclonal antibodies to liposomes bearing the same maleimide derivative (Heath et al., 1983a). Conjugation requires the introduction of thiol residues on to the antibody by reaction with succinimidyl 3-(2-pyridydithio)propionate and mild reduction with dithiothreitol at pH 4.5 (Carlsson et al., 1978). The latter method is now most frequently used in this laboratory as it is both efficient and can be applied to any

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