

INVOLVEMENT OF PLASMA PROTEINS IN LIPOSOME- HEPATOCYTE INTERACTIONS

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Adsorption of plasma proteins to liposomes

While in circulation, liposomes readily adsorb a vast collection of proteins. Some of these plasma proteins may act as opsonins, directing liposomes to cell surface receptors and promoting their uptake by macrophages in liver and spleen. Although in literature on liposome opsonization emphasis is mostly on the role of macrophages and macrophage-directed opsonins, from previous studies in our laboratory a role has emerged for “hepatocyte-directed opsonins”. The major goal of this research project was to evaluate whether such “hepatocyte-directed opsonins” do exist. We analysed the proteins adsorbing to the surface of differently composed liposomes during incubation with human or rat serum. The protein adsorption patterns of uncharged neutral liposomes and negatively charged liposomes containing phosphatidylserine (PS) (10% and 30%) or phosphatidylglycerol (PG) (10% and 30%) were compared. Strongly negatively charged liposomes containing 30% PS or PG adsorbed much more protein than uncharged neutral liposomes, which is consistent with observations by Chonn *et al.* [**J. Biol. Chem.** 267 (1992) 18759].

Role of apolipoprotein H (β_2 -glycoprotein I)

One protein, with a molecular weight of around 55 kDa, adsorbed abundantly to liposomes containing 30% PS or PG. The protein was identified by Western blotting as β_2 -glycoprotein I (β_2 GPI), also known as apolipoprotein H. Chonn *et al.* [**J. Biol. Chem.** 270 (1995) 25845] observed that the circulation time of negatively charged liposomes was prolonged after pretreating mice with anti- β_2 GPI antibodies, suggesting a role of β_2 GPI in the clearance of these liposomes. However, our *in vitro* uptake and binding experiments with HepG2 cells, isolated rat hepatocytes, Kupffer cells and liver endothelial cells using liposomes preincubated with β_2 GPI showed no enhancing effect of this protein on the association of negatively charged liposomes with any of these cell types. On the contrary, in most cases even an inhibitory effect was observed, which increased with increasing negative charge of the liposomes. These data indicate that the rapid elimination of negatively charged liposomes from circulation by any of these liver cell types is unlikely to be mediated by adsorption of β_2 GPI. Earlier studies in our laboratory have shown that binding of negatively charged liposomes containing PS to cells is likely to be mediated by charge-charge

interactions. Therefore, the massive adsorption of β_2 GPI to liposomes containing 30% PS/PG may mask the PS headgroup, leading to decreased cellular uptake and binding of these liposomes.

Role of apolipoprotein E

The role of apolipoprotein E (apoE) in the clearance of neutral and negatively charged liposomes by hepatocytes was assessed in apoE-deficient mice. We demonstrated that the uptake of uncharged liposomes by hepatocytes is nearly exclusively apoE-mediated. Negatively charged liposomes, on the other hand, although adsorbing substantially larger amounts of apoE than neutral liposomes, are cleared by mechanisms *not* involving apoE. Since apoE is a high-affinity ligand for the low density lipoprotein (LDL) receptor, which plays an essential role in high-affinity hepatic clearance of lipoprotein remnant, we also evaluated the involvement of this receptor in the elimination of neutral and negatively charged liposomes from the blood. Pretreating rats with 17α -ethinyl-estradiol, an agent known to upregulate hepatic LDL receptor expression in this animal, failed to increase liposomal uptake, which might be taken as an argument against a role of this receptor in the apoE-mediated elimination of liposomes.

Our data imply that, depending on liposome composition, the uptake of liposomes by a particular cell population is determined by the absorption of one or more specific plasma proteins (e.g. apoE). Specific protein binding may effectively influence the accumulation of certain liposome formulation(s) in a particular cell type while being ineffective for others. For example, the uptake of uncharged liposomes by hepatocytes is apoE-mediated, while that of negatively charged liposomes containing PS, which adsorb even more apoE than neutral liposomes, is not. Adsorbed proteins, such as β_2 GPI, may also act as “non-specific dysopsonins” which may hinder the interaction of liposomes with specific opsonin(s) or shield the recognition sites of specific opsonins by cell surface receptors.

Selective transfer of liposomal constituents

Liposomes or liposomal constituents are generally internalized by phagocytes or non-phagocytic cells via receptor-mediated endocytosis. Although not receiving much attention, additional mechanisms, such as selective lipid transfer, have been suggested to play a role in the elimination of certain liposomal constituents as well. In an earlier study [Verkade, H.J. *et al.* **Biochem. J.** 284 (1992) 259] we showed that *N*-rhodamine-phosphatidylethanolamine (*N*-Rh-PE) is eliminated from plasma several-fold faster than phosphatidyl- 14 C]choline, when the two labels are present together in small unilamellar vesicles (SUV) containing PS. The *N*-Rh-PE label was shown to be preferentially taken up and processed by hepatocytes. Since during *in vitro* incubation of liposomes with plasma the *N*-Rh-PE remained chemically intact and tightly associated with the liposomes, our observations suggested that the *N*-Rh-PE label was selectively

removed from the liposomes during their transient interaction with the hepatocytes (and not already in the blood).

Involvement of scavenger receptor B type 1 (HDL receptor)

The potential role of scavenger receptor class B type I (SR-BI) on the hepatocyte surface in this elimination process is described. SR-BI, also known as HDL receptor, is abundantly expressed on cholesterol-processing cells, including hepatocytes. It mediates the transfer of a variety of lipids between lipoproteins and cells. Besides lipoproteins, also plain lipid vesicles can serve as lipid donors for SR-BI-mediated selective lipid transfer. Moreover, SR-BI binds PS-containing liposomes. Using transfected Chinese hamster ovary (CHO) cells over-expressing SR-BI we showed that SR-BI enhances cell association of *N*-Rh-PE from negatively charged liposomes containing PS, whereas there is no effect of SR-BI on the cellular uptake and binding of the *N*-Rh-PE from neutral liposomes. The latter observation is compatible with the notion that negatively charged liposomes are ligands for SR-BI while neutral liposomes are not. Uptake of *N*-Rh-PE label from PS-containing liposomes by CHO cells over-expressing SR-BI exceeded that of [³H]-cholesterylolelyl ether ([³H]-COE), when the two labels were both present in the liposomal bilayers, while uptake by control CHO cells was the same for the two labels. These observations indicate the involvement of SR-BI in the selective uptake of liposomal *N*-Rh-PE by cells. The preferential uptake of *N*-Rh-PE over [³H]-COE was further enhanced by apolipoprotein A-I (apoA-I), which has been reported to be involved in the selective lipid transfer from HDL to cells. Based on these observations, we propose that SR-BI serves as a receptor mediating a transient interaction between negatively charged liposomes and the cell membrane, thus initiating the selective transfer of liposomal constituents to the cell membrane.

In vivo pharmacokinetics of liposomal constituents

In a separate study we investigated the liposomal parameters that may influence the differential pharmacokinetics of liposomal lipid constituents. Three lipophilic labels, i.e. *N*-Rh-PE, [³H]-COE and [¹⁴C]-phosphatidylcholine ([¹⁴C]-PC), were incorporated into liposomes and the pharmacokinetics of these liposomal labels in rats were compared, using liposomes of different sizes and compositions. Liposome size and cholesterol content are shown to be important parameters in the occurrence of selective elimination of *N*-Rh-PE from both neutral and negatively charged liposomes. The enhancing effect of an increase in cholesterol content (from 40% to 50%) on the selective elimination of the *N*-Rh-PE is likely to be accounted for by a bilayer destabilizing effect of excess cholesterol, reducing its potential to accommodate *N*-Rh-PE. The effect of size on the selective elimination of *N*-Rh-PE may result from the stronger curvature of the smaller liposomes which might add to the propensity of *N*-Rh-PE to leave the liposomal bilayer in favor of an acceptor membrane.

In summary, we showed that plasma opsonins play a critical role in the *in vivo* clearance of liposomes by hepatocytes. The effect of specific opsonin(s) on liposome-hepatocyte interaction, however, depends on liposome composition. The *in vivo* fate of a certain liposome formulation(s) cannot be predicted simply based on the amount of total protein or a particular type of protein bound to liposomes. Besides endocytosis, selective lipid transfer plays an additional role in the elimination of certain liposomal constituents, and SR-BI is likely to be involved.