

TARGETING LIPOSOMES AND LIPOPLEXES TO CELLS IN THE LIVER

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The liver harbors several populations of cells that play a crucial role in a number of vital functions of the body. In addition, this organ is often a primary site of metastatic tumor growth often leading to catastrophic consequences for the patient.

We have studied the potential of lipid-based drug carriers to deliver therapeutically active agents specifically to the following four liver-associated cell populations: hepatocytes (parenchymal cells), Kupffer cells (resident liver macrophages), sinusoidal endothelial cells and intrahepatically growing colon carcinoma cells.

Hepatocytes

For the delivery of substantial amounts of liposomal material active targeting to this cell type is by no means a necessity. Several groups of investigators, including ourselves, have reported on attempts to actively target liposomes to the hepatocytes, mostly by exploiting the asialoglycoprotein receptor which is abundantly present on this cell type.

We have shown however, that also without such active targeting substantial delivery of systemically administered liposomes to hepatocytes can be achieved. The smaller the liposome size, the larger the fraction that is delivered to the hepatocytes. This is probably a direct consequence of the presence of numerous open fenestrations in the endothelial lining of the sinusoids, which have an average diameter of approximately 150 nm. Small liposomes with diameters below 100 nm have relatively easy access to the transendothelially located hepatocytes. However, also liposomes with diameters even surpassing those of the endothelial fenestrations may gain access to the hepatocytes in large quantities, depending on their lipid composition. Particularly in studies based on microscopic observations the significant contribution of the hepatocytes to liposome elimination is frequently overlooked. The almost 100-fold larger mass of the hepatocyte population as compared to the Kupffer cell population (10-fold larger volume per cell and almost 10 times as many cells) will cause a microscopically visualized marker to appear much more concentrated in the

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Kupffer cells than in the hepatocytes. As a result, accumulation of marker in the latter population is usually considered negligible as compared to that in the Kupffer cells.

Currently, we are studying mechanisms of interaction of liposomes with hepatocytes, in particular the involvement of plasma proteins and lipoprotein receptors. There appear to be remarkable differences in interaction and uptake mechanism between differently composed liposomes. Apolipoprotein E strongly enhances uptake of neutral liposomes, while uptake of charged liposomes is barely influenced by this protein, despite a higher binding capacity. ApoH (β 2-glycoprotein I) on the other hand binds strongly to negatively charged liposomes (30% PS), but appears to have little effect on (in vitro) uptake by either hepatocytes, Kupffer cells or endothelial cells.

In conclusion: a substantial fraction (in some cases >50%) of an injected dose of liposomes can reach and be taken up by the hepatocytes without the need for active targeting. As a matter of fact, in many cases the attachment of (ga)lactosyl moieties on the liposomal surface even causes an increase in uptake by the liver macrophages at the expense of the hepatocytes.

Although plasma proteins may clearly affect the interaction of certain liposomes with hepatocytes, there seems to be no simple correlation between the amounts of that protein binding to the liposomes and the extent to which these interact with the hepatocytes or the rate at which they are eliminated from the blood stream..

Kupffer cells

Like hepatocytes, Kupffer cells also take up a major proportion of an injected dose of liposomes from the blood circulation, almost irrespective of the liposomal composition. This efficient form of passive targeting has been exploited for example by delivering antimicrobial agents or macrophage-activating factors like muramyl dipeptide to Kupffer cells for the control of intracellular infections such as *Leishmania* and the combat of metastatic tumor growth in the liver.

Sinusoidal endothelial cells

A third important cell population in the liver are the sinusoidal endothelial cells (LSEC) with their characteristic fenestrations. They represent approximately two thirds of the total non-parenchymal cells of the liver, the Kupffer cells accounting for the other one third.

The LSEC have been shown to be remarkably resistant to interaction with or uptake of almost any type of liposomes. However, by attachment of poly-aconitylated human serum albumin (Aco-HSA) to the liposomal surface we were able to re-direct a major fraction of an injected dose of liposomes to this cell population. This uptake process was shown to be scavenger receptor-mediated. Recently we showed that also a lipid-coated oligodeoxynucleotide/cationic lipid lipoplex could be efficiently targeted to the endothelial cells by means of surface

attachment of Aco-HSA. In this way, about one third of an injected dose of oligonucleotide could be delivered to this cell type.

Metastatic tumor cells

Another liver-associated population of cells we have tried to address with liposomes are colon carcinoma cells metastatically growing in this organ. For that purpose we attached a tumor-cell specific monoclonal antibody (CC52) to the liposomes and injected those immunoliposomes intravenously into rats carrying liver metastases of a syngeneic CC531 colon adenocarcinoma. The antibody was coupled in either one of two ways to the liposomes, one in which it was randomly thiolated and coupled to bilayer-anchored MPB-PE via the thiol groups and the other in which the antibody was attached exclusively via its Fc moiety to the distal end of bilayer-anchored PEG-DSPE. Although there were significant differences between these two immunoliposome types in terms of pharmacokinetic behaviour, uptake into the tumor nodules was not significantly different. Besides, both liposome types showed high uptake by macrophages *in vitro*. Inhibition experiments with poly-inosinic acid and aggregated immunoglobulin indicated that both scavenger and Fc receptors are involved in the uptake process. Thus, even the attachment of the antibody to the liposomal surface in a strictly oriented manner, by means of its Fc moiety, failed to prevent recognition by the Fc receptors on the macrophages. The only modest advantage these latter liposomes over the randomly coupled ones is that, *in vitro* and at relatively low antibody densities, the uptake by the macrophages was lower while recognition and binding by the tumor target cells was higher than that of the randomly coupled liposome formulation, resulting for the Fc-coupled liposomes in a tumor-cell:macrophage uptake ratio in favor of the tumor cells. All other liposomal formulations clearly favored the macrophages over the tumor cells.

In our tumor model, however, even this advantage will probably not be exploitable. We found by silver-enhancement microscopy of tumor-bearing livers of rats injected with gold-containing CC52-immunoliposomes hardly any gold associated with tumor cells. Most gold-containing cells in the tumor areas were tentatively identified as macrophages. In addition, liposome-derived gold was occasionally observed in necrotic areas. This lack of specific uptake of the immunoliposomes by tumor cells in the metastatic nodules is attributed partly to the effective competition by tumor-associated macrophages and partly to limited accessibility of the tumor cells because the tumor cells in these nodules were observed to form tightly packed clusters.

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