Liposomes: bags of challenge

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The major problem in chemotherapy is the targeting of drugs to restricted anatomical sites and specific target cells. Many drugs produce side effects, are toxic to normal tissues and are often prematurely inactivated or excreted. To overcome such difficulties, several forms of drug carriers have been described and tested in animals, and on some occasions in man (Ryman & Tyrrell, 1980; Gregoriadis, 1981). Among these drug carriers, liposomes, being versatile non-toxic and biodegradable, have received considerable attention as carriers of various drugs such as antitumour agents, antibiotics, hormones, proteins and DNA (Ryman & Tyrrell, 1980; Patel & Ryman, 1981).

The term ‘liposome’ was first proposed by Bangham et al. (1965). They consist of one or more concentric lipid bilayers enclosing an equal number of aqueous compartments in which water-soluble substances can be entrapped. Various techniques have been described to prepare liposomes of different sizes and characteristics (Ryman & Tyrrell, 1979; Szoka & Papahadjopoulos, 1981).

Liposomes have been administered by various routes (topically, parenterally, by inhalation and orally), and this subject has been reviewed by Patel & Ryman (1981). Subcutaneously, intraperitoneally and intramuscularly injected liposomes are drained from the circulation via the lymphatics. In lymph, some liposomes are degraded and some reach the circulation intact. Thus liposomes can be used via these routes for (i) delivery of drugs to lymph nodes (Khat et al., 1982) and (ii) detection of metastatic spread in lymph nodes by encapsulating lymphoscintigraphic material (Osborne et al., 1980). Oral administration of liposomes can survive digestion in the stomach, although they are degraded in the intestine. However, they protect the entrapped proteins from proteolytic digestion and facilitate their absorption from the gastro-intestinal tract (Patel & Ryman, 1981).

Intravenously injected liposomes are rapidly sequestered by mononuclear phagocytes of the reticuloendothelial system. Their blood clearance rate depends on the charge, size and lipid composition of liposomes. In the circulation, their properties such as permeability, size and surface charge are altered rapidly, and they can be broken down by lipoproteins, circulating phospholipases or by complements. (Patel & Ryman, 1981). By selecting the composition of liposomes, the stability of the vesicles in the circulation can be improved (Gregoriadis et al., 1983). In blood, liposomes may be taken by circulating leucocytes and monocytes and interact with platelets and endothelial cells lining the capillaries. Liposomes do not escape from the circulation to tissues other than those with discontinuous endothelial lining to their capillaries, i.e. liver and spleen. In these tissues, phagocytic cells of the reticuloendothelial system remove the vesicles which are degraded by lysosomes.

The fact that liposomes are predominantly taken up by the tissues rich in reticuloendothelial cells limits the use of liposomes in cancer chemotherapy, as cytotoxic drugs in liposomes will destroy cells of liver and spleen. Cancer patients require a healthy reticuloendothelial system to clear the toxic products of cancer cells and control the spread of the disease to normal tissue. Furthermore, owing to the lack of penetration of intact liposomes through capillary endothelial barriers, liposomes may not reach many diseased tissues. These factors will also limit the use of antibody-labelled liposomes to target to specific cells in vivo.

Thus the original hope that the liposome would present a new form of drug carrier with greater selectivity and the potential for targeting to specific tissues has not been, and may never be, realized. However, it seems that passive targeting, the natural ‘homing’ of liposomes to elements of the reticuloendothelial system, may be exploited in several ways.

Passive targeting to the reticuloendothelial system

(i) Delivery of drugs or immunomodulators to the reticuloendothelial system. (a) Poste and Fidler and their colleagues (Fidler et al., 1981; Poste & Fidler, 1982) have carried out elegant work in which they have shown that the immunomodulator muramyl dipeptide, when entrapped in liposomes and injected intravenously, is taken up by circulating monocytes, which then become activated alveolar macrophages. The activation of macrophages by immunomodulator results in control of lung metastases induced in the experimental animal model. This technique can be used to introduce other immunomodulators or biologically active substances in monocytes. However, the efficiency of the uptake of liposomes by monocytes requires some improvement to activate enough macrophages for the total arrest of metastases.

(b) Liposomes with an appropriately entrapped drug have been used selectively to attack parasites, parts of whose life cycle involves the reticuloendothelial system (Alving & Stock, 1979). Thus leishmaniasis, schistosomiasis and malaria may all prove to be more effectively treated by liposome-entrapped drugs than by the free drugs currently in use against these diseases.

(ii) Rapid removal of toxic substances from the circulation. Toxic concentrations of drugs or metabolites may be removed from the circulation by injecting an antibody directed against the toxic agent, e.g. digoxin-digoxin antibody (see Fig. 1). However, difficulties exist in that the high back-ground radioactivity in blood and other body fluids owing to the radiolabelled antibody to the tumour product (primary antibody) causes lack of definition on the scans. However, Begent et al. (1982) have shown that this difficulty can be overcome to a certain extent by administering an antibody (i.e. secondary antibody) attached to liposomes to the primary antibody (for further details, see Barratt et al., 1984).

The concept of using antibody-labelled liposomes to remove toxic substances or for radioimmunodetection of tumours is very promising, but the success of this strategy depends on the development of a technique of coupling immunoglobulin G to the liposome surface so that the liposome-immunoglobulin-G complex is stable in the circulation. The coupling should be specific (see Fig. 2), so that
Digoxin Antibody to digoxin
\[ \text{Antibody-digoxin complex in circulation} \]

Digoxin Liposome-antibody Digoxin-antibody-liposome complex
\[ \text{Liver and spleen degradation} \]

Fig. 1. Removal of digoxin from circulation by using antibody–liposome complex

Random association
(a) \[ A = \text{Enrapsed antibody} \]
\[ B = \text{Surface-associated antibodies} \]

Specific association (b and c)
(b) Antibodies attached to liposome surface via F(ab)\(_2\)

(c) Antibodies attached to liposome surface via Fc portion of the molecule: increased antigen-binding capacity.

Fig. 2. Antibody attachment to liposomes

(a) Random association: poor antigen-binding capacity. (b) Specific association: antibody attached to liposome surface via F(ab)\(_2\), facilitates the binding to Fc receptors on the cell membrane. (c) Antibody attached to the liposome surface via Fc portion of the molecule: increased antigen-binding capacity.

the maximum number of antibody molecules become available for antigen binding. The problem of aggregation of antibody-bound liposomes also requires solving.

Considering the drug-delivery aspect of liposome-carriers, the uptake of circulating liposomes by blood monocytes and reticuloendothelial cells in liver and spleen is a major obstacle in the targeting of liposomes to other cell types and tissues. Attempts to decrease the uptake by reticuloendothelial cells have been made by 'blockading' the phagocytic uptake mechanisms by colloidal particles such as dextran sulphate or carbon particles (Souhami et al., 1982), or by predosing animals with 'empty liposomes' followed by a second therapeutic test dose of liposomes (Abra & Hunt, 1981).

Another strategy is to manipulate liposome composition so that they have a lower affinity for the reticuloendothelial system. Patel et al. (1984) have shown that the uptake of liposomes by the reticuloendothelial-rich tissues liver and spleen is inhibited by increasing the cholesterol content of liposomes. However, neither of the above approaches is very successful in diverting liposomes away from macrophages, and, even if this goal is achieved, the major problem of reaching the target cells by escaping through the endothelial lining of the capillaries in extravascular tissues has still to be considered.

Thus liposomes, once described by Ryman (Ryman & Tyrrell, 1980) as 'Bags of Potential', now appear to be more like 'Bags of Challenge'.


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Interaction of liposomes with hepatocytes and Kupffer cells in vivo and in vitro

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The dominant role of the liver in the clearance of intravenously injected liposomes and the relative contribution of the various liver cell types to the uptake and processing of the vesicles has gained considerable attention during recent years (Roerdink et al., 1981; Rahman et al., 1982; Poste et al., 1982).

With large-size multilamellar vesicles containing radioiodinated bovine serum albumin as a marker for the encapsulated aqueous space, we found, after intravenous injection, that most of the label rapidly accumulated in the liver, mainly in the sinusoidal-cell fraction (Scherphof et al., 1983). After reaching a maximum about 20 min after injection, the label content of the cells declined, owing to intracellular proteolysis and release of low-molecular-weight degradation products. With the non-degradable \( ^{125} \)I-labelled poly(vinylpyrrolidone) as an aqueous space marker, we found, after separating the isolated liver sinusoidal cells in a Kupffer-cell fraction and endothelial-cell fraction by elutriation centrifugation, that most of the radioactivity was present in the Kupffer cells, whereas the endothelial cells did not contain significant amounts (Roerdink et al., 1981).

These results were in good agreement with morphological observations on the localization of horseradish peroxidase. After injection of multilamellar vesicles containing this enzyme, exogenous peroxidase activity was localized mainly in the lysosomes of sinusoidal cells, especially Kupffer cells (Roerdink et al., 1977). Presumably, the 0.1-µm fenestrations in the endothelial cells lining the liver sinusoids (Wisse, 1970) effectively prevented access of these large liposomes to the liver parenchymal cells.

However, with phosphatidyl[\( ^{3} \)H]choline as a marker of the liposomal membrane, we found substantial amounts of radioactivity in the parenchymal cells after intravenous injection of the multilamellar vesicles, particularly at longer times after injection (Roerdink et al., 1981). In fact, we observed a shift of \(^{14} \)C radioactivity from the non-parenchymal cells to the parenchymal cells, suggesting an intercellular communication between the two cell populations in the liver, the nature of which has still to be elucidated.

With small unilamellar vesicles (diameter 25-80 nm) we found, using \(^{3} \)Hinulin as an aqueous-space marker, after intravenous injection, uptake of the liposomes by both Kupffer and parenchymal cells (Roerdink et al., 1983). Uptake by the hepatocytes was substantial, but, on a per-cell base, significantly lower than that by the Kupffer cells. It seems likely that these small-size vesicles reach the hepatocytes by penetrating the endothelial barrier through the fenestrations.

By comparing the fates of \(^{3} \)Hinulin and \(^{14} \)Cphosphoglyceromyelin as markers of the encapsulated volume and of the lipid phase respectively, we observed intrahepatic liposomal lipid degradation with subsequent release of watersoluble degradation products. Release of degradation products occurred especially from Kupffer cells rather than from parenchymal cells, which tended to retain the \(^{3} \)H and \(^{14} \)Clabels to equal extents. In both cell types we also found substantial conversion of labelled sphingomyelin into labelled phosphatidylcholine. From these results we concluded that the intracellular fate of the liposomal lipid is cell-type-dependent: after intracellular degradation of the lipid, the Kupffer cells partly release the water-soluble degradation products and partly re-utilize them for synthesis of cellular phosphatidylcholine de novo, whereas the hepatocytes efficiently re-utilize all of the degradation products. The lack of release of degradation products from the hepatocytes is in line with our observation that in this cell type the conversion of sphingomyelin into phosphatidylcholine occurred very efficiently.

Experiments with the lysosomotropic agent chloroquine provided a clue to the mechanism of uptake of the vesicles by the liver cells after intravenous injection (Table 1). Treatment of the animals with chloroquine before liposome

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### Table 1. Effect of chloroquine treatment of rats in vivo on the conversion of liposomal \(^{14} \)Cphosphoglyceromyelin into phosphatidylcholine in parenchymal and non-parenchymal liver cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Treatment</th>
<th>Sphingomyelin</th>
<th>Phosphatidylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenchymal</td>
<td>None</td>
<td>14.2</td>
<td>82.7</td>
</tr>
<tr>
<td>Parenchymal</td>
<td>Chloroquine</td>
<td>47.2</td>
<td>47.5</td>
</tr>
<tr>
<td>Non-parenchymal</td>
<td>None</td>
<td>62.3</td>
<td>37.5</td>
</tr>
<tr>
<td>Non-parenchymal</td>
<td>Chloroquine</td>
<td>90.1</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Rats were injected intravenously with small unilamellar vesicles (5 µmol of total lipid/100 g body wt), composed of \(^{14} \)Ccholine-labelled sphingomyelin/cholesterol/phosphatidylserine (4:5:1). At 2 h and 1 h before and at 1 h after injection of the liposomes, the animals were treated with chloroquine or received 0.9% NaCl. At 2 h after liposome injection parenchymal and non-parenchymal cells were isolated and extracted with chloroform/methanol. Lipids were fractionated by t.l.c. and analysed for radioactivity.