

LIPOSOMES IN DRUG DELIVERY: STRUCTURE, BEHAVIOUR IN VIVO AND APPLICATIONS

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Synopsis

Many phospholipids, alone or in combination with other lipids (including lipid extracts from membranes), will form liposomes.

The successful evolution of liposomes from an experimental tool to industrially manufactured products for clinical and veterinary use depends on efficient drug entrapment in vesicles of a narrow size distribution using simple, reproducible and inert methods. Encouraging results with liposomal drug in the treatment or prevention of a wide spectrum of diseases in experimental animals and humans have reinforced the view that clinical applications may be forthcoming.

Riassunto

Molti fosfolipidi da soli o in combinazione con altri lipidi danno luogo a formazione di liposomi. L'evoluzione della tecnica dei liposomi dall'uso di laboratorio all'utilizzazione industriale per prodotti adatti sia per l'animale che per l'uomo dipende dalla loro capacità di intrappolare farmaci in modo stabile ed uniforme e con metodiche facilmente riproducibili.

I recenti risultati ottenuti, sia sugli animali che sull'uomo con l'uso clinico di farmaci liposomiali per il trattamento di una vasta gamma di patologie fanno intravedere un uso clinico sempre più diffuso di questo nuovo tipo di veicolo.

Liposomes: Structure and properties

Phospholipids and other polar amphiphiles form closed concentric bilayer membranes (liposomes or vesicles) when confronted with excess water with each bilayer representing an unbroken bimolecular sheet (lamellae) of lipids (1). In the process of their formation liposomes entrap water and solutes if present. Alternatively, lipid soluble agents and molecules coupled to lipids can be incorporated into the liposomal membrane. Thus, almost any substance, regardless of solubility, size, shape and electric charge can be accommodated in liposomes as long as there is no interference with their formation (1).

Many phospholipids, alone or in combination with other lipids (including lipid extracts from membranes), will form liposomes. Depending on their gel-liquid crystalline transition temperature (T_c - the temperature at which hydrocarbon regions change from a quasicrystalline to a more fluid state), phospholipids determine bilayer fluidity and stability in terms of permeability to solutes *in vitro* and *in vivo*. Bilayer fluidity and stability can also be influenced by the inclusion of sterols (for example cholesterol). The incorporation of charged amphiphiles will not only render the liposomal surface positively or negatively charged but also increase the distance and hence aqueous volume (and solute entrapment) between bilayers (1).

The unusually versatile nature of liposomes, which was established by membrane biologists who used them as a model for cell membrane studies prompted the development of another, perhaps more exciting, concept (2): the use of the system in targeted drug delivery.

Progress in this area with a wide range of liposomal drugs (for example anti-tumour and anti-microbial agents, enzymes, hormones, vitamins, metal chelators, genetic material, immunomodulators and vaccines) has been rapid

and a vast amount of information has been obtained, already (1).

Liposome technology

The successful evolution of liposomes from an experimental tool to industrially manufactured products for clinical and veterinary use depends on efficient drug entrapment in vesicles of a narrow size distribution using simple, reproducible and inert methods (3). In this respect, there has been considerable success and well defined formulations containing a variety of active agents can now be produced in a stable form. A number of these formulations are currently undergoing clinical trials (1,4) and a few are already licensed. However, several of the methods developed, although highly efficient, have the drawback of being uneconomical, of being applicable only to drugs of low molecular weight (thus excluding vaccines, enzymes and other proteins) or requiring the use of detergents, sonication or organic solvents (3). These may, in turn, be detrimental to the structure-activity relationship of certain drugs, especially macromolecular agents.

In a recently reported technique (5,6), which is both simple and easy to scale up, high yield entrapment of drugs in dehydration-rehydration vesicles (DRV) occurs under mild conditions. Entrapment values for a number of drugs, antigens and immunomodulators in DRV were substantial and reproducible. Protein-containing DRV can be freeze-dried in the presence of a cryoprotectant and most of the protein content is retained within intact vesicles on reconstitution with saline. Moreover, microfluidization of DRV leads to the formation of smaller (about 100nm in diameter) vesicles retaining much of the originally entrapped drugs (6). Because of the limited number of steps involved in most methods of liposome preparation, sterility of the starting materials can easily be maintained using aseptic techniques.

Behaviour of liposome in vivo

Many workers (1,3,4,7) with diverse research interests have administered drug-containing liposomes to animals and humans, parenterally and enterally. As a result, much is now known of their behaviour. Of particular interest are (a) the effect of components of biological fluids, with which injected liposomes first come into contact, on the retention of liposomal structural integrity and (b) the rates at which liposomes are cleared from the site of administration and distributed among the tissues, mostly within macrophages of the reticuloendothelial system (RES). In both cases, the behaviour of liposomes is dictated by their structural characteristics. For instance, plasma high density lipoproteins (HDL) will remove phospholipid molecules from the bilayers of intravenously injected conventional liposomes, for example those made of egg phosphatidylcholine (PC). These will then disintegrate and release their drug contents. By substituting PC with 'high melting' phospholipids (for example distearoyl phosphatidylcholine (DSPC) $T_c=54^\circ\text{C}$) or supplementing phospholipids with excess cholesterol bilayers become rigid at 37°C or have their phospholipid molecules packed and, therefore, resistant to HDL attack. Thus, liposomal integrity is preserved and entrapped drugs remain with the carrier en route to its destination.

It is now established that the more stable the liposomes, the lower their rate of clearance from the blood circulation (1). It is suggested that liver-specific opsonins, implicated in the removal of liposomes from the circulation by the RES (principally in the liver), do not adsorb as avidly on vesicles with rigid or packed bilayers. The relationship between liposomal stability and clearance is altered when a negative or (under certain conditions) a positive surface charge is imposed on the bilayer surface, with even the most long-lived liposomes assuming short half-lives. A similar reduction in half-

life occurs as vesicle size increases; this may be partially reversed by coating liposomes with hydrophilic molecules. Not surprisingly, liposomes with extended half-lives are deposited in the RES at reduced rates, with a considerable proportion (about 30% for small unilamellar vesicles) favouring the macrophages of the bone marrow. When these liposomes are small enough they will also gain access to the hepatic parenchymal cells through the fenestrations. Regardless of whether uptake is mediated through opsonins or other ligands, it occurs through endocytosis although fusion may also be involved to some extent (1).

While such findings on stability, clearance and tissue distribution relate to liposomes injected intravenously, they also concern preparations given by alternative parenteral routes such as intraperitoneal, subcutaneous and intramuscular (1): a proportion of liposomes, determined by vesicle size, composition and route of injection, enters the lymphatic and eventually, the blood circulation where they behave as if given intravenously. However, whereas liver, spleen and bone marrow take up nearly all liposomes given by the intravenous route, they will account for a smaller proportion of the dose given by other routes. The remainder (up to about 80% of liposomes injected subcutaneously or intramuscularly) is retained at the site of injection and attacked by infiltrating macrophages or other factors, or intercepted by the lymph nodes draining the injected site. Relative to their mass, uptake by lymph nodes is much greater (over 100-fold) than that by any of the other RES tissues.

Substantial efforts to ascertain whether liposomes given enterally enhance absorption of agents which are either not absorbed by, or unstable in the gut, have given inconclusive results. In spite of indications that insulin, factor VIII, anticoagulants and vitamins administered via liposomes do reach the blood circulation, their absorption is unpredictable and only mini-

mal. It is nevertheless apparent that liposomes of a lipid composition (for example cholesterol-supplemented phospholipids with high Tcs) that renders them resistant to detergents or phospholipase attack, protect agents from gut enzymes. Such liposomes may survive the gut milieu to some extent and thus facilitate the absorption of their contents, probably through the lymphatics (1).

Targeting of liposomes

Delivery of liposomal drugs to cells that do not normally take up the carrier effectively has been achieved by antibodies and other cell-specific ligands covalently or hydrophobically linked to the outer bilayer of liposomes. In vitro studies (1) have repeatedly demonstrated that polyclonal or monoclonal antibodies raised against a repertoire of cell surface antigens mediate the association of the drug-containing liposomal moiety (to which such antibodies are linked) with, and its introduction into, the respective cells. However, in vivo targeting of liposomes has proved a much more challenging proposition (1), especially when mediated via antibodies, the Fc portion of which binds to its receptors on the macrophage, thus accelerating removal of the carrier by the RES. Circumvention of this problem has been achieved by the use of the antigen-recognizing Fab portion of

the immunoglobulin molecule as a ligand or by taking advantage of the already long half-lives of small, stable vesicles. In the latter case, Fc-mediated shortening of the half-life of vesicles will still allow them to circulate long enough for targeting to occur. Such complications, however, do not occur when certain galactose-, mannose-, and fucose-terminating glycoprotein and glycolipid ligands are used, since these will associate exclusively with their receptors in vivo (1).

Implications in medicine

Encouraging results (1,4,7) with liposomal drugs in the treatment or prevention of a wide spectrum of diseases in experimental animals and in humans have reinforced the view that clinical applications may be forthcoming. These include treatment of skin diseases, skin care, antimicrobial therapy, metal chelation, enzyme and hormone replacement therapy, vaccines (7) and diagnostic imaging. To that end, the first and obvious consideration is that a liposomal drug preparation designed to treat a particular disease should have clear advantages over the conventional use of the therapeutic agent. Recently, progress toward clinical uses of liposomes has gained new momentum thanks to the efforts of related biotechnology companies.

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