Nano- or Submicron-Sized Liposomes as Carriers for Drug Delivery

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Liposomes are tiny spheres ranging in diameters from 50 nm to several microns. The scope of this mini review is to introduce the concept of liposomes and to describe some aspects and mechanisms of stimulating topical and injectable products with liposomes. Two examples discussed in this article are topical delivery across skin and injectable formulations for anticancer drugs. Classic liposomes are of little value as carriers for drug delivery via the skin because they do not penetrate it deeply. Only specially designed liposomes have been shown to be capable of achieving enhanced delivery. The incorporation of additives, such as anionic surfactants and ethanol, can fluidize the phospholipid bilayers, thus increasing the depths to which liposomes can penetrate the intercellular pathways of the skin. Also, liposomes that have been conjugated with PEG or antibodies can increase the residence time of anticancer drugs in the circulation and enhance drug accumulation in tumors. *(Chang Gung Med J 2006;29:358-62)*

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Over the last 20~25 years, liposome-encapsulated drug delivery has undergone intense investigation. During this period, considerable amounts of experimental data have been generated and successful enhancement of a diverse array of molecules has been achieved. These thermodynamically stable, lamellar structures form spontaneously when lipid is brought into contact with an aqueous phase. Unlike micelles and lipid emulsions, liposomes have an entrapped, discontinuous aqueous phase separated by bilayered lamellae from the continuous aqueous phase. It is recognized that the phenomenon of spontaneous compartmentation might be used to improve the therapeutic index of drugs by delivering the active ingredient to the appropriate site of action.[1]

The skin is our largest organ and forms a fascinating and unique interface between the outside world and us. It is accessible and relatively easy to interrogate in vivo. Advances in drug delivery into and through the skin have established a unique and useful method among the various routes of drug administration. Applying medication to the skin is an instinctive method that has played a significant role in the history of primitive remedies without clear exploration. The nature and essence of this simple-looking but rather complicated delivery method should be further clarified into transdermal delivery and topical administration. A limited number of drugs can be formulated as topical or transdermal delivery products due to the functions of the stratum corneum (SC), which provides the principal barrier to skin permeation. One of the possibilities for increasing skin permeation of drugs is the use of liposomal systems.[2] Only specially designed vesicles have been shown to be capable of allowing enhancement.[3]

The development of a drug carrier system would also be expected to reduce adverse effects and

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increase the efficacy of antitumor agents by controlling drug movements in the body. Liposomes represent useful antitumor drug carriers via injection, in that they prevent extensive distribution in the body and are capable of carrying encapsulated drugs in a selective manner to target tissue.[4] The scope of this mini review is to introduce the concept of liposomes and to describe some aspects and mechanisms of stimulating topical and injectable products with liposomes.

Liposome structures

Liposomes have been extensively investigated as a potential drug delivery system due to the enormous diversity of structure and composition that can be achieved.[5] Liposomes are microscopic vesicles consisting of one or more membrane-like phospholipid bilayers surrounding an aqueous medium. After the appropriate preparation procedures, the vesicle size can range from 50 nm to several hundred nm. Due to their high degree of bio-compatibility, liposomes have been used as delivery systems for an assortment of compounds. The lipid components of liposomes are predominantly phosphatidylcholine (PC) derived from egg or soybean lecithins. The minor components in lecithin are phosphatidylserine, phosphatidic acid, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol.[6] Due to their biphasic character, liposomes can act as carriers for both lipophilic and hydrophilic drugs. Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal environment and exhibit different entrapment and release properties. Lipophilic drugs are generally entrapped almost completely in the lipid bilayers of liposomes. Since they are very poorly soluble in water, problems like loss of entrapped drug on storage are minimal with this class of drug. Hydrophilic drugs may be entrapped inside the aqueous core of liposomes. However, it is also possible that hydrophilic drugs are located in the external water phase. The percentage of hydrophilic drug encapsulated by liposomes depends on the liposome bilayer composition and preparation procedure.

Application of liposomes for topical drug delivery

Liposomes can increase drug delivery via skin routes by different mechanisms (Fig. 1). One rationale for including liposomes in drug formulations is that liposomes may act as permeation enhancers by penetration of individual lipid components. Phospholipids are able to diffuse into the SC, and the interactions and enhancer effects of liposomes on the SC are based on the lipid mixing of liposomal phospholipids with lipid bilayers of the skin.[7] Phospholipids in liposomal systems can disrupt the bilayer fluidity in the SC, decreasing the barrier properties of the skin. Moreover, some investigators report that phospholipids in liposomes may mix with the SC lipids creating a lipid-enriched environment.[7,8] This lipid depot in the skin is preferred by lipophilic drugs, resulting in enhanced skin uptake. In some cases, phospholipids themselves can be the solubilizers to increase the solubility of lipophilic drugs such as indomethacin and miconazole.[9]

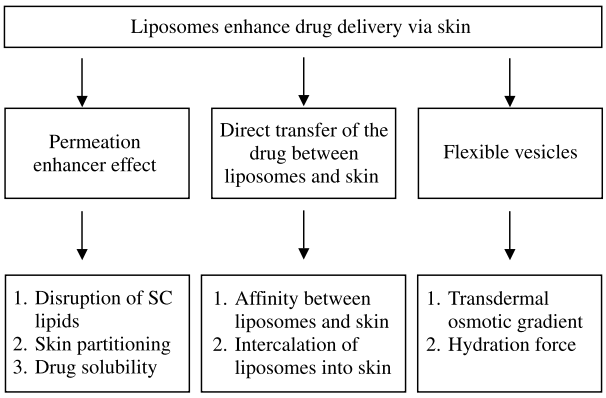

Fig. 1 Proposed mechanisms of liposome enhancement of drug delivery via the skin.

Phospholipids should exhibit their enhancing effect on skin in the presence of organic solvents such as propylene glycol, tetraglycol and ethanol.[10] The concentration of phospholipids and the presence of unsaturated fatty acids in phospholipids are also important factors affecting transdermal flux of drugs.[8-10] Egg PC (EPC), soybean PC (SPC) and dioleylphosphatidyl ethanolamine (DOPE) increase the drug permeation into the skin, and distearoylphosphatidylcholine (DSPC) did not change or increase it. EPC, SPC and DOPE have low value gel-liquid crystalline phase transition temperature (T_c) and they are in fluid-state at skin temperature of 32°C. It seems that fluid-state phospholipids disturb the rigid bilayer structures of skin lipids leading to increased drug partitioning into the lipid phase.

The mechanisms of the SC-liposome interaction have been studied by many investigators using differential scanning calorimetry (DSC), X-ray diffraction, electron paramagnetic resonance (EPR), fluoromicrography and confocal laser scanning microscopy. Most of the investigators have suggested that direct transfer of the drug between the phospholipid bilayers of liposomes and the lipid content of the skin causes the enhancement of drug delivery via skin (Fig. 1). In this case, liposomes do not appear to function as permeation enhancers but provide the needed physicochemical environment for transfer of drugs into the skin.

The most controversial theme relating to the topical use of liposomes is whether intact liposomes are able to penetrate into the SC or even down to the deeper strata of the skin. Ho et al. assumed that the liposomal vesicles were neither absorbed intact nor fused with the SC surface, after performing an in vitro study using mouse skin.[11] Lasch et al. found that intact liposomes are confined to the SC and do not penetrate into deeper skin layers.[12] However, the so-called transfersomes, the specially designed lipid vesicles, are not only able to penetrate the SC but are also able to reach the systemic circulation due to transdermal osmotic gradient and hydration force.[13] The diversity of the results of these investigations may be due to the different liposomal systems and evaluating methods used. Liposome penetration into skin depends greatly on lipid composition, the thermodynamic state of the bilayers and presence of ethanol in the formulation. The key for liposome penetration into skin is the liquid or gel state of the vesicles. The liquid-state vesicles may act not only in superficial SC layers but may also induce lipid perturbations in deeper layers of the SC, while gel-state vesicles always interact only with the outmost layers in the SC. Elastic and rigid vesicles show similar definition. Elastic liposomes can be produced after incorporating single-chain lipids, surfactants or ethanol into the lipid bilayers.[13] When drugs remain strongly associated with the vesicles, elastic vesicles can be used to transfer drugs rapidly into the deeper layers of the SC, after which the drugs can permeate into the viable epidermis. Elastic vesicles have superior characteristics compared to rigid conventional vesicles, both in terms of drug permeation and skin interaction. Some factors can influence skin permeation of drugs encapsulated in liposomes. Table 1 shows these possible factors.

Table 1. Physicochemical Factors of Liposomes Themselves Affect the Skin Permeation of Drugs

Physicochemical factor	General trend to enhance drug permeation
Thermodynamic activity	liquid state > gel state
Electrostatic charge	a controversy
Size	small > big
Lamellarity	unilamellar layer > multilamellar layers

Application of liposomes for tumor targeting

Several factors limit the efficiency of drug distribution to tumors. First, drugs will predominantly distribute into well perfused tissues such as liver and kidneys, with relatively low concentrations in poorly perfused tissues such as tumors. Second, low molecular weight compounds, if not bound by proteins, are readily excreted in the urine by glomerular filtration. Third and last, protein binding of many cytotoxic drugs is usually rapid, leading to decreased drug bioavailability and, in many cases, irreversible drug inactivation.[14] As a result of a variable combination of these factors, the pool of circulating free drug available for tumor uptake is significantly diminished, affecting the prospects of therapeutic efficacy. The ability of liposomes to localize effectively to tumors is somewhat enigmatic. Even in the absence of tumor cell-specific ligands attached to their surface, liposomes will localize to tumors. In this sense, it is generally accepted that liposomal tumor targeting is a passive function and depends largely on the number of times that an individual liposome passes through the vascular network within a tumor.

The blood vessels in a tumor are abnormally leaky as a result of significant structural and functional anomalies. This leakiness, together with the co-existing lack of a fully functional system of lymphatic drainage, is thought to account for the extravasation and retention of liposomes within the tumor interstitium. Therefore, by altering the physicochemical properties of liposomes, the ability to remain in circulation can be altered.[15] For some applications, liposomal carriers have been limited by their marked tendency to localize in the tissues of the mononuclear phagocyte system (MPS), particularly the liver and spleen. Thus classical liposomes, along with their associated drugs, are often eliminated from circulation by cells of the MPS before effective

delivery.[16] The shortcomings of classical liposomes can be overcome by the development of liposomes bearing surface carbohydrates, such as monosialoganglioside, or polymers, such as polyethylene glycol (PEG). These liposomes circulate for longer periods of time than classical liposomes.[17]

Conjugated liposomes are not readily taken up by the macrophages in the reticuloendothelial system (RES) and hence stay in circulation for a relatively long period. PEG is particularly useful because of its ease of preparation, relatively low cost, controllable molecular weight and linkability to lipids or protein including the antibody by a variety of methods, compared to monosialoganglioside.[17,18] Long-circulating liposomes composed of PEG2000 with an average diameter of 100~200 nm were found efficiently accumulated in tumors such as colon cancer.[19] The results indicate that the size of long-circulating liposomes were an important factor for extravasation. Due to the increased circulation time of the liposomes containing PEG2000 and the leaky structure of microvasculature in the solid tumor tissue, these liposomes have been shown to accumulate preferentially into the tumor tissue. Thus, under physiological tumor conditions, only small liposomes ranging from 100 to 200 nm with prolonged circulation half-life encounter more opportunities to extravasate through discontinuous capillaries, as well as to escape the gaps between adjacent endothelial cells and openings at the vessel termini during tumor angiogenesis. Doxil®, which is the drug doxorubicin encapsulated in PEG-liposomes, has been approved in the USA and Europe. The approval was for treatment of AIDS-related Kaposi's sarcoma, ovarian cancer and breast cancer.

The presentation of multiple targeting molecules on the surface of individual liposomes can restore multivalent binding of monovalent antibody fragments and hence increase their binding avidity for the target antigens. These are the so-called "immunoliposomes". Another advantage of immunoliposomes lies in the potential for additivity or synergy between immunoliposomes, the signaling antibodies present at the liposome surface and the encapsulated drugs. In animal xenograft studies of human cancers, therapeutic antibodies showed additive or even synergistic activities when used in combination with chemotherapeutic drugs.[20] Clinical studies have demonstrated additive benefits for an antibody against anti-HER2 (Herceptin®) in combination with taxol or in combination with anthracycline drugs plus cyclophosphamide.[21] Immunoliposomes can be targeted using monoclonal antibodies that have unique signaling properties, such as inhibition of DNA repair, induction of cell cycle arrest, blockade of P-glycoprotein or induction of apoptosis, which lead to anticancer effects that may synergize with the cytotoxic effects of the liposomal anticancer drugs.[20]

Conclusions and future prospects

Many studies have verified the enhancing efficiency of liposomes on topical or injectable drug delivery. However, most of the studies cited in this mini review focused on in vitro or laboratory experiments. Future challenges for skin or tumor applications of liposomes are therefore the urgent need to establish in vivo and clinical data. Confirmation of liposomal use in clinical and in vivo systems may extend their applicability. Although liposomes are theoretically thermodynamically stable, conquering the stability problem during storage is an important issue. The instability of liposomes can cause leakage of encapsulated drugs from the vesicles and aggregation and/or fusion to form larger vesicles. The definitive nano-sized vesicles may not exhibit their benefits after aggregation. Progress in academic investigations may be modified to become commercial patents and products of the future. Although advanced attempts have successfully been applied in the laboratory, many problems need to be overcome before they can be commercialized. Determining how to change or modify preparation procedures from laboratory scale to mass production is also important. Resolving these realistic difficulties is the challenge and mission for future development of new products and formulations related to liposomes.

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