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Liposomes as Carriers of Antimicrobial Drugs

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ABSTRACT

Liposome are promising drug carrier systems being developed. Their successful use in the treatment of several diseases demonstrates that a solid rationale for clinical development of liposomes as antimicrobial drug carriers can be established. There are a number of potential drug candidates for liposome encapsulation. The involvement of several biotechnology companies has culminated in the design and licensing of formulations for the treatment of certain microbial infections and cancers. Understanding of liposome behavior in the body and of the physicochemical mechanisms involved in the interaction of liposome, drug, and cellular targets is essential for their future applications.

Liposomes are microscopic vesicles consisting of multiple concentric lipid bilayers formed when an aqueous solution is added to a dried lipid film. They were used initially as models of biological membranes; more recently, great interest has been generated in their use as drug cariers. The interest was probably based on their biocompatibility and presumed lack of toxicity. They have been extensively used in an attempt to improve the therapeutic index in known active drugs.

In the last decade a variety of novel methods have been proposed to improve drug delivery. Among these, the use of phospholipid vesicles or liposomes has received much attention, principally as a method to prolong drug levels following injection or to direct drugs to specific sites in the body. The advantage of liposomes as drug delivery systems stems from the biocompatible nature of the lipids used to form them and the ability to prepare them easily in various sizes and compositions (1).

In the selection of liposomes as drug carriers, major factors that should bee considered are the liposome's size, charge, membrane fluidity, and biodegradability. Size and charge will definitely affect the distribution and uptake of liposomes. Although liposome particles are cleared mainly by macrophage-phagocyte system, larger particles may be trapped in capillaries. Negatively

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charged, fluid liposomes are preferentially taken up by macrophages. The incorporation of sterols usually increases the rigidity and stability of liposomes.

Another important consideration is the selection of phospholipids; selection should be based on their biocompatibility and lack of toxic effects. Phospholipids such as phosphatidyl choline (PC), phosphatidyl etanolamine (PE), and phosphatidyl glycerol (PG), which are present in most mammalian cell membranes, seem good choices for liposomal formulations (2).

By varying the lipid composition of the liposomes, it is possible to manipulate the rate of intracellular capsulated agent. Less-fluid liposomes, by virtue of the highly packed acyl chains in the lipid membranes, are less susceptible to lysosomal enzyme activities than are fluid liposomes, causing a delay in liposomal degradation after uptake of the vesicles by macrophages. In this respect, the macrophages serve as depot cells from which the drug is released during a prolonged period of time. The fluid liposomes are composed, for example, of cholesterol, phosphatidylcholine, and phosphatidylserine (PS), which have a transition temperatures well below 37°C. Less-fluid liposomes are composed of cholesterol, distearoylphosphatidylcholine (DSPC), and dipalmitoylphosphatidylglycerol (DPPG). Slow degradation of the less-fluid liposomes in vivo resulted in a decrease in the therapeutic effect of the antibiotic (3).

Unilamellar and large multilamellar liposomes did not exhibit large differences in their efficacy, and similar quantities of drugs may have been delivered to the intracellular sites of infection, although higher tissue levels were achieved with multilamellar liposomes. Multilamellar liposomes may have the added advantage of not leaking all of their contents when the outer bilayer is exposed to serum components, whereas unilamellar liposomes may lose more of their contents during such an encounter. Previous studies have shown that unilamellar liposomes (prepared by reverse-phase evaporation) remain in circulation longer than multilamellar liposomes, while the latter are removed by the spleen and liver more efficiently. Some advantages of unilamellar liposomes include their amenability to filter sterilization and their uniform size distribution, which render them more suitable as eventual pharmaceuticals (4).

Administration of liposome-encapsulated antibiotics and antivirals has resulted in enhanced efficacy against a number of infections localized in the reticuloendothelial system, including those due to *Leishmania donovani, Listeria monocytogenes, Candida albicans, Brucella abortus*, and Rift Valley fever virus (4–6).

Liposomes are taken up by macrophages in vitro and in vivo, and liposome-encapsulated antimicrobial agents show greater killing of intracellular bacteria, including MAC (*Mycobacterium avium* complex) in macrophage cultures in vitro than do nonencapsulated antimicrobial agents.

One of the major rationales for the use of liposomes to deliver antimicrobial agents involves targeting the drug to intracellular infections of the reticuloendothelial system. Such targeting is possible because of the natural uptake of liposomes and other particulates by these tissues. The actual amount taken up by these organisms is somewhat dependent on the dose of lipid administrated. With small doses, most intact liposomes are retained by the liver. With larger doses, the ability of the liver to clear the particles from the circulation may be overwhelmed, and the amount remaining in the circulation that can be taken up by the spleen and bone marrow thus increases. Still larger doses can produce a temporary reticuloendothelial system blockade which results in a longer circulation time but eventually a similar tissue distribution (7).

Lipoproteins and lipid vesicles inhibited toxicity of heptaene polyenes (Candidin, Candicidin, Mepatricin A) and nystatin to mammalian cells, but not to fungal cells. These polyenes bind more avidly to ergosterol than to cholesterol. In contrast, lipoproteins and lipid vesicles equally affected the toxicity of nonheptaenes (Filipin, Etruscomycin, Fungichromin, Natamycin) to mammalian and fungal cells. These polyenes do not preferentially bind to ergosterol (7).

ANTIMICROBIALS

Streptomycin

Since the discovery of streptomycin in 1944, the aminoglycosides have had a major role in the therapy of mycobacterial diseases. Although streptomycin continues to be an important drug in treatment regimens for tuberculosis, its use in the treatment of infection due to nontuberculous mycobacteria is not well defined. Other aminoglycosides such as kanamycin and the cyclic peptide capreomycin appear to have limited value in the treatment of nontuberculous mycobacterial disease. Liposome encapsulation of aminoglycosides appears to be a promising approach to the treatment of mycobacterial infections (1,4,8–10).

Liposome-encapsulated streptomycin treatment of mice infected with *Mycobacterium tuberculosis* resulted in prolonged survival and decrease in numbers of organ-

isms recovered from the spleen compared with treatment with free streptomycin. Liposome-encapsulated streptomycin is superior to free streptomycin in the intracellular killing of *Staphylococcus aureus* (9).

It is possible that alternate methods of targeting streptomycin or other antibiotics to the lungs, such as aerosolization of free or encapsulated drug, will be more effective than administration of multilamellar liposomes (4).

Gentamicin

Antimicrobial activity of gentamicin (4,8) has not been well studied. Although it has been found to have good in vitro antituberculous activity by the broth dilution method, gentamicin showed poor activity compared with streptomycin in murine tuberculosis model. Gentamycin activity against organisms in the spleen and liver was markedly enhanced by liposomal encapsulation.

Treatment with liposomal preparation at 20 mg/kg produced a 1.5-log reduction in viable cell counts after only 5 days of therapy. In each experiment except the prophylaxis study, liposomal encapsulation failed to enhance the activity of gentamicin against organisms in the lung. It is unclear why the liposome-encapsulated gentamicin did not have stronger sterilizing activity for the spleen and liver (8).

Liposome-encapsulated gentamicin prepared by Liposome Company (Princeton, NJ) is currently in clinical trials (4). It would be considered for further evaluation in the treatment of MAC infection in humans.

Rifampin

The antitubercular activity of rifampin was considerably increased when it was encapsulated in egg phosphatidylcholine liposomes (4). A further increase in the activity was observed when the macrophage activator tetrapeptide tuftsin was grafted on the surface of the drug-loaded liposomes. Intermittent treatments (twice weekly) with these preparations were significantly more effective than the continuous treatments. Rifampin delivered twice weekly for 2 weeks in tuftsin-bearing liposomes was at least 2000 times more effective than the free drug in lowering the load of lung bacilli in infected animals. However, pretreatment with drug-free tuftsin-bearing liposomes did not render the pretreated animals resistant to the *Mycobacterium tuberculosis* infections; neither did it appreciably increase the chemotherapeutic efficacy of the liposomized rifampin. These results clearly demonstrate that liposome targeting to macrophages could considerably increase the antitubercular activity of liposomized drugs such as rifampin. Also, it shows that immunoprophylactic treatment with macrophage activators such as tuftsin does not afford any advantage in treatment of tuberculosis infections, presumably because of inactivation of the primed macrophages by the mycobacterial sulfatides.

Clarithromycin and Olfoxacin. Liposome-encapsulated clarithromycin may be more effective than the free form of the drug against MAC infections in vivo, and the use of a combination therapy with ethambutol could further enhance the efficacy. Liposome entrapment of either ofloxacin or clarithromycin significantly enhanced the activities of the drugs, when compared with the antimycobacterial effects of equivalent concentrations of the free drugs (4,5).

Ampicilin

Liposomal entrapment of ampicillin resulted in an increased availability of the antibiotic for the intracellular bacterium *L. monocytogenes* in murine macrophages. At an ampicillin concentration of 12 mg/ml intracellular bacterial killing was absent when ampicillin was encapsulated in the less-fluid liposome type; however, when encapsulated in fluid liposomes, ampicilin was still bactericidal at that concentration. The observation that intact liposomes containg ampicillin were not bactericidal for *L. monocytogenes* indicates that intracellular killing of bacteria is the result of cellular uptake of liposomes followed by lipsomal release of ampicillin intracellulary (3).

Amikacin

A liposome-encapsulated formulation of the aminoglycoside antibiotic amikacin was effective against MAC (usually associated with chronic pulmonary disease in nonimunocompromised patients. and disseminated infection in patients with AIDS) in the livers, kidneys, and spleens of beige mice, while the free antibiotic at the same dose was ineffective. Liposome-encapsulated amikacin was also effective against MAC in human monocytes and in murine peritoneal macrophages (4,7).

Depending on the chemical structure and charge, liposomes are promptly phagocytized by mononuclear phagocytes and provide high intracellular concentrations of the drug with minimal toxic effect. Negatively charged large unilamellar and multilamellar vesicles containg amikacin at relatively low doses had enhanced efficacy in the treatment of early MAC infection compared with

similar doses of the free drug (11). The bactericidal effect was maximal at 48 hr after treatment, and cultures from macrophage lysates 72 hr after treatment did not show greater killing. The liposomes prepared were relatively stable. Liposome-encapsulated amikacin retained 81.2% of its antibiotic after 24 hr and 68% of drug after 72 hr (9).

Tobramycin

Liposomal delivery of local antibiotics may be clinically useful in surgical wound prophylaxis, and the advantages include the achievement of adequate local antimicrobial concentration at the time of operation, as well as increased efficacy of the antibiotic (12,13,21). The liposomal delivery of local antibiotics in this model of surgical wound infection reduced the number of organisms more effectively than locally applied free drug. Treatment of contaminated surgical wounds is often complicated by the failure of local or systemic antibiotic treatment and prophylaxis. Locally administered liposome-encapsulated antimicrobials may offer advantages over free antibiotics, including an increase in efficacy, ease of administration, and safety. The local delivery of agents by liposomes may also have advantages in other areas such as oncology, wound healing, immunology, and cellular biology. The therapeutic advantages, as well as the absorption and distribution of locally administered liposome-encapsulated antibiotics, were compared with those of locally applied unencapsulated antibiotics in a contaminated wound model. Liposomes can potentially alter toxicity and target drug delivery to specific sites. In addition, they may permit the use of lipophilic drugs that would otherwise be difficult to administer systemically.

Pulmonary infection was associated with a lowering of tobramycin levels in lungs. Encapsulation of tobramycin in liposomes can result in a significant increase of its residence time within lungs.

Tobramycin is a good candidate for liposome aerosol delivery against cystic fibrosis infection.

Resistant tumors may be sensitive to anthracyclines delivered by liposomes. Local administration of liposome-encapsulated antibiotics in osteomyelitis would be expected to achieve high local drug levels and reduce the dose of systemically administrated antibiotics.

Amphotericin B

Amphotericin B (AmB) is a polyene macrolide antibiotic that displays a broad spectrum of activity against most fungal pathogens (1,14–20,22–27,29–32). Aspergillosis, a severe life-threatening fungal infection, is more prevalent in immunodebilatated patients suffering from tuberculosis, cancer, AIDS, etc. AmB is the only potent drug of choice for the therapy of this infection. The mechanism of action of AmB is related to its affinity for sterols in biological membranes—this affinity accounts for the formation of transmembrane pores that lead to the release of vital intracellular constituents (5). The antifungal activity of drug lies in its preferential binding to sterol, forming a transmembrane channel and leading thereby to release of vital metabolites from the fungal cell and causing ultimate death of fungus. However, the interaction of AmB with mammalian cells, especially red blood cells, makes it very toxic; thus use of this drug as a therapeutic measure is restricted (10). Its administration results in a variety of side effects (severe, chills, nausea anemia, etc.) which limit the clinical application of AmB.

Liposomes have been used as vehicles for AmB in treatment of murine histoplasmosis, cryptococcosis, and candidiasis. Results in the treatment of infections caused by the filamentous fungi were less impressive. The best results were in the treatment of hepatosplenic candidiasis, an infection especially common among patients with acute leukemia. even though in vitro studies show equivalent antifungal effectiveness for both AmB and L-AmB, reported in vivo experience in both animal and human studies shows much more favorable results with liposomal amphotericin B, with reduction in toxicity. It appears that injected liposomal AmB is distributed primarily to organs of the reticuloendothelial system, where AmB is transferred directly from the liposome to the ergosterol-containing fugrale wall, thus reducing toxicity to other cholesterol-containing mammalian cells. This affinity for organ rich in reticuloendothelial cells should make liposomal AmB especially useful in treating fungal infections that involve the liver, spleen, lung, and bone marrow. The rationale for the work on AmB was that because both the parasites and liposomes were taken up by phagocytic cells, the AmB entrapped in the liposomes might be brought into close proximity to the parasite inside the host macrophages (18).

Liposomal AmB is a promising improvement over the current formulation of the drug. It is obvious that a number of details concerning liposome preparation and storage influence the extent of the reduction of lethality. AmB intercalated in liposomes is less toxic than, but as efficacious as, the drug solubilized in deoxycholate. Lopez-Berestein et al. (14,15) observed that the lipid composition played a major role in liposomal AmB ac-

tivity. L-AmB preparations composed of phospholipids alone were shown to be more effective than those containing ergosterol or cholesterol. A possible explanation for these findings was that the sterol-containing L-AmB preparations combined AmB more tightly, reducing the potential for drug interaction with fungal membranes. When the lipid composition was held constant, the size of vesicles had an appreciable effect on LD_{50}. For the sterol-containing preparations, the lethality increased as a vesicle size increased. We would anticipate that SUV (small unilamellar vesicle) generally would be less toxic than MLV (multilamellar vesicle) preparations. The decrease in toxicity when SUV are the carrier is suggested to be due to the reduction in the rate of transfer of the drug from the liposome into cellular membranes, and not to a major redistribution of the drug in the organs of the body tissues. It has been shown that amphotericin B incorporated into small unilamellar liposomes retains the good inhibitory and fungicidal activity of free amphotericin B (9).

AmBisome, Amphocil, and ABLC are formulations which incorporate amphotericin B, licensed for use in certain European countries and elsewhere including the United States.

CONCLUSIONS

Liposome are promising drug carrier systems being developed. Their successful use in the treatment of several diseases demonstrates that a solid rationale for clinical development of liposomes as antimicrobial drug carriers can be established. There are a number of potential drug candidates for liposome encapsulation. Most of products have been developed on the basis that they show a proven reduction in toxicity and a more favorable distribution of the formulated drugs into target areas. Some of the resulting formulations are already licensed for use, some are awaiting approval, and others are in clinical trials. Understanding of liposome behavior in the body and of the physicochemical mechanisms involved in the interaction of liposome, drug, and cellular targets is essential for their future applications.

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