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# Liposome-based drug delivery to alveolar macrophages

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The recent development of liposomal formulations compatible with aerosol delivery has expanded the potential to utilise chemotherapeutic agents directly targeted to the lungs more effectively. These are agents that would otherwise not be used because of their low solubility or toxicity. Various properties of liposomal carriers, including size, surface charge, composition and the presence of ligands, alter their efficacy and specificity towards alveolar macrophages to a great extent. This editorial summarises the advances in liposome-based drug delivery to alveolar macrophages.

Keywords: alveolar macrophages, amphotericin B, fungal infections, liposomes, tuberculosis

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## 1. Introduction

Although drugs to combat a wide range of genetic, malignant and infectious diseases are available, their efficacy is often compromised by their inability to reach target sites at an appropriate concentration. Consequently, much attention has been focused on the use of drug delivery systems, which are expected to optimise the action of the drugs already in existence by targeting or by facilitating their release where they are needed.

Effective chemotherapy can be practically implemented in pulmonary infections using liposomes, through drug targeting to alveolar macrophages (AMs). However, therapeutic applications of intravenously injected liposomes have been limited due to several factors, such as leakage of their contents into the plasma compartment before they reach the target tissue, rapid clearance from the bloodstream and their uptake by the macrophages of the liver and spleen. Pressurised packed or aerosolised liposomes for pulmonary targeting of drugs have been well documented [1,2]. Mannose receptors expressed abundantly in the liver, spleen and AMs have been most widely utilised for targeting bioactive molecules to the macrophages [3]. Drugs are released from the liposomes following intralysosomal degradation.

AMs form a first-line defence against microorganisms entering the lung via the airways. In contrast to the interstitial macrophages in the lung, AMs, which are located in the disalveolar space, have direct access to liposomes administered via the airways, for example by intra-tracheal instillations, intra-nasal administration or by the application of aerosolised liposomes.

## 2. Targeting alveolar macrophages for intracellular infections

### 2.1 Fungal infections

Fungal infections are very likely to be progressive in nature, and are often disseminated, ultimately becoming life threatening. The ability to utilise lipophilic drugs to deliver high concentrations of drug directly to the site of infection, and to reduce toxicity, makes aerosol liposomes an attractive alternative for pulmonary administration [4]. The usefulness of liposomes as vehicles for amphotericin B (Amp B) has been well demonstrated in fungal diseases, such as candidiasis, aspergillosis and cryptococcosis, by reducing its toxicity and increasing its therapeutic

index. Recently, targeted delivery to lung tissues, the organs infected by many fungi, via inhaled liposomal Amp B aerosol, has been shown to be a more effective approach. Following the promising clinical results, three formulations containing Amp B (Abelcet® [Enzon Pharmaceuticals, Inc.]; Amphocil® [Three Rivers Pharmaceuticals, LLC], and AmBisome® [Gilead Sciences]) are available in the market. AmBisome is a liposomal formulation of Amp B for injection, developed by NeXstar Pharmaceuticals (acquired by Gilead Sciences in 1999). It is marketed by Gilead in Europe and licensed to Astellas Pharma (formerly Fujisawa Pharmaceuticals) for marketing in the US, and to Sumitomo Pharmaceuticals for marketing in Japan. Fungizone® (Bristol-Myers Squibb) is a liposomal complex of Amp B, and is the latest and cheapest addition to the lipid-based formulations of Amp B, with many reported advantages. It is marketed by Lifecare Innovations. Other formulations include Amphotec® (Intermune) and Abelcet. Abelcet is not a liposomal preparation, but rather a lipid complex preparation.

A variety of Amp B-lipid formulations were tested for the optimal treatment regimen for Cryptococcus and Candida infections in mouse models. AmBisome retained its anticryptococcal activity even when animals were challenged 14 days after aerosol treatment. At day 21, when organisms had spread to the brain in all animals, the single 2-h aerosol treatment reduced the cryptococci load in the brain as well as in the lungs, showing the potential of liposomes in treating both local, pulmonary cryptococcal disease and systemic disease [5]. In a Candida–mouse model, systemic candidiasis and mortality were reduced by aerosolised Amp B–liposome treatment [6].

Nebulised liposomal Amp B was studied in a steroid-immunosuppressed murine model of invasive pulmonary aspergillosis, with subjects showing a significantly improved survival rate compared with subjects administered with intravenous deoxycholate Amp B [7]. In addition, the effects of treatment with aerosolised Amp B desoxycholate and aerosolised liposomal Amp B were evaluated. Treatment with aerosolised liposomal Amp significantly prolonged survival with all the treatment regimens when compared with other subjects. The effects of Amp B desoxycholate and liposomal Amp B on pulmonary surfactant function were also evaluated *in vitro*. Amp B desoxycholate inhibited surfactant function in a dose-dependent fashion. Liposomal Amp B had no detrimental effect on the surface activity of the surfactant [8]. Nebulisation of Amp B desoxycholate (Fungizone) and liposomal Amp B (AmBisome) leads to respirable aerosols, and results in a substantial lung tissue concentration of Amp B with low systemic exposure [9].

Furthermore, to increase the scope of liposomised Amp B, various macrophage-specific ligands, such as *O*-palmitoyl mannan and *O*-palmitoyl pollulan, were tested to dock the liposomal constituents specifically to the target cells actually in need of treatment. Pressurised packed systems based on preformed liposomal formulations in chlorofluorocarbon, aerosol propellants were evaluated for their targeting potential to AMs. The drug localisation index, calculated after 6 h, was approximately 1.42-, 4.47- and 4.16-fold higher, respectively, for plain *O*-palmitoyl mannan- and *O*-palmitoyl pollulan-coated liposomal aerosols, when compared with plain drug solution-based aerosols [10]. Also, tuftsin-bearing liposomes encapsulating Amp B were studied for their efficacy against *Aspergillus fumigatus* infections in mice. The percentage survival of *A. fumigatus*-infected mice considerably increased by 70 – 75%, and the surviving animals were virtually free of any fungal load [11].

Systematic evaluation of multilamellar vesicles (MLV) with different phospholipid composition reveals that certain classes of phospholipids are recognised preferentially by macrophages. Inclusion of negatively charged phospholipids, such as phosphatidylserine and phosphatidylglycerol in MLV consisting of phosphatidylcholine, greatly enhances their binding to macrophages and their phagocytosis by macrophages. In contrast, neutral MLVs composed exclusively of phosphatidylcholine are not efficiently bound to macrophages. Such enhancement of phagocytosis and specificity to macrophages has been shown to occur in mouse peritoneal macrophages, mouse Kuppfer cells, rodent AMs, human peripheral blood monocytes and human AMs [12]. The effect of charge on the disposition characteristics of aerosolised liposomes was reportedly evaluated using a Collison nebuliser. Mice were exposed via the nose to Amp B-containing liposomal aerosols of positive, negative or neutral surface charge characteristics. Amp B was not detected in serum or in other organs, such as kidneys, liver and the brain. The deposition of neutral and positive liposomal Amp B in lungs followed biexponential kinetics. The $\alpha$ and $\beta$ phase $t_{½}$ for positive liposomes were 1.3 and 15.1 days, respectively, and 2.3 and 22.0 days for neutral liposomes. Amp B delivered as negatively charged liposomes exhibited monoexponential clearance with a $t_{½}$ of 4.5 days, clearly indicating the potential of such systems for long-term protection against fungal infections [13].

The influence that the particle size of ciprofloxacin-loaded liposomes developed for treatment of respiratory intracellular parasite infections has on drug delivery to rat AMs, following pulmonary administration, was also studied. Prepared liposomes were adjusted to five different particle sizes (100, 200, 400, 1000 and 2000 nm). Following pulmonary administration of liposomes with average particle size 1000 nm, the concentrations of drug in rat AMs until 24 h later were significantly above the minimum inhibitory concentration of ciprofloxacin, when tested against various intracellular parasites. Furthermore, in a cytotoxic test, following pulmonary administration of ciprofloxacin liposomes with an average particle size of 1000 nm, no release of lactate dehydrogenase (LDH) was observed from rat lung tissues, indicating efficient delivery of ciprofloxacin to AMs without any cytotoxic effects on lung tissues [14].

### 2.2 Tuberculosis

Among various forms of tuberculosis, pulmonary tuberculosis is the most common, with the involvement of lung macrophages containing a large number of tubercle bacilli. Effective chemotherapy for pulmonary tuberculosis can be attained by targeting drugs to lung tissue by tagging specific markers or homing devices onto the surface of liposomes. Liposomes, as well as delivering drugs to the infected site, could also act as drug reservoirs to provide a slow and sustained release of the drug. Rifampicin-loaded aerosolised liposomes were evaluated for their selective presentation to AMs, the most dense site of tuberculosis infection. Egg phosphatidylcholine- and cholesterol-based liposomes were modified by imparting negative charge (using dicetylphosphate) or by coating them with alveolar macrophage-specific ligands (maleylated bovine serum albumin [MBSA] and *O*-steroyl amylopectin [*O*-SAP]). The percentage viability of *Mycobacterium smegmatis* inside macrophages (*in vitro*) after administration of drug (*in vivo*) was estimated to be in the range of 7 – 11% for ligand-anchored liposomal aerosols, and it was recorded to be 45.7 and 31.6% for plain drug and plain, neutral liposomal aerosol (based on phosphatidylcholine:cholesterol) treated macrophages, respectively. Results suggest preferential accumulation of MBSA- and *O*-SAP-coated formulations in the lung macrophages, which was further reflected in the periodically monitored *in vivo* tissue distribution studies. Ligand-anchored liposomal aerosols are not only effective in the rapid attainment of a high drug concentration in the lung (population of AMs), but also in maintaining this over a prolonged period of time [15].

In another study, rifampicin delivered twice-weekly for 2 weeks in tuftsin-bearing liposomes, was found to be significantly more effective than free drug in lowering the load of lung bacilli in infected animals [16]. Modifying the liposomal surface with *O*-SAP imparts lung specificity, and the lipid derivative of PEG provides reticuloendothelial system (RES)-avoiding characteristics to liposomes. In addition, the encapsulation of antituberculous drugs in the liposomes resulted in the reduction of toxic side effects. These observations suggest that encapsulation of antitubercular drugs in lung-specific stealth liposomes will certainly improve chemotherapy against human pulmonary tuberculosis [17].

### 2.3 Other intracellular infections

Aerosolised liposomal pentamidine appears to be an effective therapy for *Pneumocystis carinii* pneumonia in rats, and produces significantly lower extrapulmonary drug deposition than parenteral administration. The severity of *P. carinii* involvement at the time of treatment influences both the level of drug delivery to the lung and the response to aerosolised pentamidine therapy [18]. Aerosol administration of liposome-encapsulated ciprofloxacin by jet nebulisation resulted in significantly higher drug levels and prolonged drug retention in the lower respiratory tract compared with the free drug. Aerosol inhalation, given either prophylactically or therapeutically, provided complete protection against a pulmonary lethal infection model of *Francisella tularensis* [19] in mice.

## 3. Conclusion

The lungs provide a large absorptive surface area, an extremely thin, absorptive, mucosal membrane and good blood supply. The noninvasive nature of this pathway makes it especially valuable for the delivery of drugs. Pulmonary, intracellular parasitic infections remain the most common form of disease, and the development of methods for delivering antiparasitic drugs directly to the lungs via the respiratory route is a rational therapeutic goal. The obvious advantages of inhaled therapy include direct drug delivery to the diseased organ, targeting to AMs harbouring the pathogens, a reduced risk of systemic toxicity and improved patient compliance. The combination of liposomes and aerosols has been utilised to directly target the lungs with chemotherapeutic agents that might not have been used because of low solubility or toxicity. Several key issues, such as patient education, cost of treatment, stability and large scale production of drug formulations, need to be addressed before inhaled therapy finds its way from theory to clinical reality, particularly in the case of tuberculosis.

## 4. Expert opinion

There are a variety of antibacterials, antifungals and antivirals that possess good *in vitro* activity, but are not effective because of their systemic toxicity and/or poor penetration into the lungs. Incorporation of many lipophilic drugs into liposomes decreases their toxicity without affecting effectiveness, thus increasing the therapeutic index. This editorial focuses mainly on the aerosol delivery of Amp B for the treatment of pulmonary and systemic fungal diseases. Liposomal systems offer distinctive advantages, such as a high loading capacity and the possibility of controlling size and permeability, and, therefore, controlling the release kinetics of the drugs from the carrier system. Aerosol administration of liposome-encapsulated drugs by jet nebulisation has delivered significantly higher drug levels and prolonged drug retention in the lower respiratory tract compared with the free drug. Liposomes tailored for size, lipid and charge composition also influence their uptake by AMs. Uptake of liposomal contents by AMs significantly increases when ligands capable of interacting specifically with surface receptors of macrophages are incorporated. Liposomal encapsulation significantly reduces the systemic activity of inhaled immunomodulators and causes a higher activation of AMs [20], and hence this approach can be used in cancer chemotherapeutics in alveolar regions. Also, compounds such as the diamidines show considerable toxic effects *in vivo*, despite their antimicrobial, antitumoural and antiviral activities. Therefore, a drug delivery approach, such

as that of the liposome-mediated macrophage 'suicide' technique, could reduce these undesirable effects. It has been further shown that both intracellular clodronate and intracellular propamidine induce apoptotic cell death in macrophages [21]. Thus, working with more effective liposome-encapsualted drugs could lead to a significant reduction in the amount of liposomal components, as evidenced in experimental models.

As the pulmonary route is noninvasive, it could become a clinically viable strategy for targeting lung-localised opportunistic and notorious infections, especially when they occur in immunocompromised patients, and particularly in the case of HIV infections. In addition, macrophage-specific targeting, especially to AMs, could eradicate intracellular parasites by addressing problems relating to multiple drug resistance, by increasing the localised manifold concentration of the drug, and by reducing contraindicated manifestations resulting from systemic drug effects. These strategies could equivocally be exploited for genetic/rDNA-based vaccine delivery. Thus, the site and targeting option in combination could offer a multitude of clinically viable strategies, which may become a great tool in the treatment and management of problematic diseases of infectious origin.



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