CHAPTER 1

INTRODUCTION

The spread of tuberculosis (TB) has reemerged as an urgent health problem. Rates for this disease have been increasing since the mid 1980s in association with the HIV epidemic. Each year, about 2 million people in the world die as a result of the infectious disease caused by Mycobacterium tuberculosis (MTB) (WHO, 2001). Current methods of treatment are far from optimal and better ones are being sought to overcome the increasing spread of TB and the problem of incompletely treated TB that contributes to the emergence of drug resistant strains. Since many patients with TB may have significant social problems, compliance with drug therapy is frequently difficult. The development of targeted drug delivery to the lungs as a means of treating TB is desirable for several reasons. Although TB is a systemic disease that can potentially affect any organ system, the lung is the major portal of entry for MTB and thereby the site of the initial immune response as well as an important site of reactivation disease (Comstock and Cauthen, 1993). Technology for lung specific drug delivery systems is now at a point where aerosols and aerosols combined with liposomes and possibly timed-release methodology may offer advantages for more effective treatment and prevention of TB.

Conventional antituberculer medications frequently have serious side effects Schreiber, 1999). Although single drugs can be effective for prophylactic treatment of skin test converters, active disease must be treated using combinations of three or four drugs over a period of at least six to nine months to insure that disease will not recur after treatment is discontinued and to prevent the emergence of resistant strains. Targeted delivery of new formulations, directly to the lungs, could result in high pulmonary levels relative to systemic levels. Thus, increasing effectiveness and decreasing toxicity. Supplementing the dose of agent delivered to the diseased lung, when it is the only clinically involved organ, could make it possible to decrease the duration of treatment in these cases. Because the systemic dose will not be increased, undesirable toxicities would be avoided. Another advantage is that this mode of delivery might make it easier to provide prolonged treatment. Improved targeted delivery approaches combined with development of new antituberculer drugs or with timed release formulations may reduce the frequency of dose delivery. This would be a major benefit in treating patients in whom it is hard to maintain effective compliance with treatment regimens. For example, longer intervals between treatments would make it easier to deliver directly observed therapy, which is an effective means of getting patients to complete a full course of treatment.

Targeting the drug to the alveolar macrophages (AMs) would be a rational addition to current tubercular therapy, potentially enhancing efficacy and reducing toxicity. Pharmaceutical aerosols, >5 μm once deposited may be removed by macrophage action before the dose is delivered, thereby reducing the bioavailability of the drug. Whereas for an antituberculer compounds it is the target region, as the pulmonary Mycobacterium tuberculosis infection is characterized by AMs containing large numbers of bacilli (Brain, 1985). Targeting the drug to AMs would be a rational addition to current therapy, potentially enhancing efficacy and reducing toxicity. Thus, a drug delivery system (Gupta and Hickey, 1991) targeted to the AMs might be effective, but has yet to be evaluated by direct administration to the lungs.

An increasing cases of drug resistant tuberculosis is common today both in tropical as well as developed countries. The first line drugs used in the treatment of tuberculosis are streptomycin, isoniazid, rifampicin and ethambutol. The second line drugs available are pyrazinamide, capreomycin and cycloserine to avoid the possibility of drug resistant. The combination therapy is widely employed involving an initial phase in which at least three drugs are used and a continuation phase where two drugs used. Depending upon the complexity of the disease the treatment paid may vary from 6-18 months.

Parenteral and more common oral administration of these drugs leads to side effects (5-7%) like hepatotoxicity, haematological changes, arthritic symptoms and toxic effects involving the central or peripheral nervous system and especially the development of resistance.

The treatment of mycobacterial infections has become more important challenging problem because of the emergence of multiple drug resistant organisms and because of Acquired Immuno Deficiency Syndrome (AIDS) pandemic, which has been associated with a marked increase in tuberculosis and infections caused by the Avieum complex. The micro-organism grows slowly and the disease often chronic, patient compliance drug toxicity and the development of microbial resistance present special therapeutic problems.

Liposomes are used as carriers for drugs and antigens. Liposomes can prolong the duration of drug exposure, acting as a slow-release reservoir. This has been demonstrated in a number of studies, for example with the antimalarial drug chloroquine or the radical scavenger superoxide dismutase (Oussoren, 2000).

Liposomes can protect a drug against degradation (for example metabolic degradation). Conversely, liposomes can protect the patient against side effects of the encapsulated drug. For example, the dose limitation of the cytotoxic drug doxorubicin is its (irreversible) damage to heart muscles. Liposomes can be used to deliver biological agents either entrapped within the internal aqueous compartments, reconstituted in the lipid bilayer, or attached to the outer surface. Liposomes are artificial lipid vesicles composed of concentric lipid bilayers that alternate with aqueous compartments. They have permeability properties similar to those of biological membranes. Liposome administration has been shown to provide delivery of antibiotics in mice infected with Mycobacterium avium (Bermudez et al, 1987; Cynamon et al, 1989; Duzgunes et al, 1988; Ehlers et al, 1996; Gangadharam et al, 1995; Leitzke et al, 1998; Nightingale et al, 1993 and Petersenet al, 1996) or M. tuberculosis (Deol et al, 1997; Orozco et al, 1986 and Vladimirsky and Ladigina, 1982) with some success. The earliest reports of liposomal administration to the respiratory tract concerned the potential replacement of pulmonary surfactant in the treatment of respiratory distress syndrome (Ivey et al, 1997). The liposomal encapsulation has been shown to reduce the entry of the agent into the systemic circulation, compared with free drug and provide distribution throughout the airspace of the lung (Juliano and McCullough, 1980). One of the major advantages of liposomes over other carrier delivery systems (Microspheres, Niosomes etc.) of drugs is that they can be prepared from materials for which there is considerable data available regarding their fate in vivo (Kellaway and Farr, 1990).

Some carrier drug delivery of rifampin vesicles in the treatment of tuberculosis in mice shows targeting to macrophages could considerably increase the activity (Agarwal et al, 1994). Encapsulation of kanamycin into liposomal vesicles increased incorporation of the drug into the host peritoneal macrophages and enhanced the antimicrobial activity (Tomioka et al, 1991). And various other workers had studied the absorption of antitubercular drugs in rat lung at various times after intra tracheal administration

Improving drug delivery to the pulmonary system has been an area of increasing interest among several disciplines. There has been extensive effort to define the factors that influence the deposition of aerosolized drug in deep lungs within the respiratory tract, clearance mechanisms from the lung, circulation in the airways, and absorption and metabolism of compounds by the lung (Chediak et al, 1990; Byron et

al, 1990). Delivering small doses of the active ingredient directly to the lung maximizes the therapeutic effect while minimizes unwanted side effects. Despite these advantages and the widespread use of therapeutic aerosols, there are several shortcomings associated with drug delivery to the respiratory tract. Although the onset of action is very rapid, the duration is often short lived as the drug can be quickly removed from the lung through various clearance mechanisms (Shenfield et al, 1976; Marriott, 1990 and Taylor, 1990).

Carrier drug delivery to the respiratory tract, whether for local or systemic activity provides an interesting challenge? The behavior of drugs in vivo can often be changed in dramatic fashion by coupling the kinetics, tissue distribution, metabolism and cellular interactions of the drug will be dictated, or at least strongly influenced by the behavior or the carrier. Judicious exploitation of these changes in pharmacodynamic behavior can lead to an enhanced therapeutic index for the drug. However, an intelligent approach to therapeutics using drug-carrier technology requires a detailed understanding of the interaction of the carrier with critical cellular and organ system.

A variety of agents have been used as drug carriers. These include immunoglobulins (Viteklta et al, 1983 and Edwards et al, 1982), Serum proteins (Poznansky and Cleleland, 1980), synthetic polymers (Chien, 1980), lipid vesicles (Juliano and Layton, 1980), microspheres (Widder and Senyei, 1983) and even cells most commonly the erythrocytes (Ihler, 1983).

The inclusion of drugs in carriers clearly holds significant promise for improvements in the therapy of several disease categories and it is confident that carrier systems will take its place, along with other drug delivery technologies, in enhancing the effectiveness, convenience and general utility of new and existing drugs (Metha et al, 1984 and Tokes et al, 1982).

With this information, strategies to improve drug delivery to the respiratory system have developed. Particulate carriers such as liposomes have many attractive features as pulmonary drug delivery systems particularly with respect to controlled delivery. Aerosolized route of administration can deliver therapeutic agents to the diseased regions while reducing their distribution to the other organs; it provides an excellent example of targeted drug therapy. Hence, a more favorable therapeutic index can be obtained for the treatment of lung diseases when drugs are administered by inhalation rather than by the oral or parenteral route. Bronchodilators, anti-inflammatory agents,

mucolytics, antiviral agents, anticancer agents and phospholipid protein mixtures for surfactant replacement therapy are all routinely given as aerosolized formulation.

A significant disadvantage of many existing inhaled drugs is the relatively short duration of resultant clinical effects and most medications in aerosol form require inhalation at least 3-4 times daily (Marriott, 1990; Taylor 1990). This often leads to poor patient compliance with the therapeutic regime and increases the possibility of associated side effects. Deposition of drugs in the desired site of lung will be particularly beneficial since drug can be delivered to and retained at the targeted site for prolonged period of time and thus can maximize therapeutic index of the drug.

Liposomes are useful tools for pulmonary delivery of drugs due to their solubilization capacity for poorly water-soluble drugs, rendering them more practical to be aerosolized. Their biodegradability allows for prolonged pulmonary residence times without danger of allergic or other deleterious side effects. The targeting capacity of infected or immunologically impaired alveolar macrophages is a unique feature of liposomes.

The use of liposome in pulmonary delivery was first investigated as a potential treatment for respiratory distress syndrome. One of the perceived benefits of liposomes as a drug carrier is based on their ability to alter favorably the pharmacokinetic profile of the encapsulated species and thus provide selective and prolonged pharmacological effects at these sites of administration. The resulting decrease in the frequency of drug dosing will significantly improve the quality of life for patients and at the same time reduce healthcare cost. The selective and controlled release of the drug is also expected top reduce or eliminate hypersensitivity and systemic toxicities. The challenging aspect still remains unanswered are the mode of delivery for liposomally encapsulated drug. Metered dose inhalers (MDI) are currently being reformulated as a result of the band being implemented throughout the world by the United nations on the use of chlorofluorocarbons (CFCs) (Juliano et al, 1986) to meet this challenge, one such alternative is the development of new and improved Dry Powder Inhaler (DPI) system that will allow inhalants administration of all drugs presently delivered with MDIs. With constrain of propellant phase out and short-term stability of liposomal aqueous dispersion the most viable alternative would be to deliver the liposomal drug in dry form.

It is assumed that problems can be minimized if not completely eliminated by concurrent administration of drugs by pulmonary route. This assumption is due to the

reason that complete elimination of the disease may require a smaller doses of drugs for less period of time, reduction in drug concentration in systemic circulation and also in the treatment of pulmonary disorders and for systemic action inhalation is the preferred route of administration. Administering liposome-encapsulated drugs by aerosols could be a feasible way of targeting drugs to the lungs, specifically to pulmonary alveolar macrophages (Myers et al, 1993). Dry powder inhalations formulation have been developed for asthma (Kawashima et al, 1998) and for deep-lung delivery of various agents (Patton, 1999 and Malcolson et al, 1998). It ahs been observed that particles reaching the lungs are phagocytosed rapidly by AMs (Evora et al, 1998). Although phagocytosis and sequestration of inhaled powders may be a problem for drug delivery to other cells comprising lung tissue, it is advantage for chemotherapy of TB. Phagocytosed microparticles potentially can deliver large amounts of drug to the cytosol than oral doses. Moreover, liposome have the potential for lowering dose frequency and magnitude, which is especially advantageous for maintaining drug concentrations and improving patient compliance. In this study, we have aimed to make Liposomal Dry Powder Inhaler (LDPI) by lyophilization process with minimal drug leakage and powder characteristic that gives improved pulmonary deposition Isoniazid (INH) and Rifampicin (RFP) are the first line antituberculer agents were selected for liposome encapsulation because, current treatment of pulmonary tuberculosis involves prolonged oral administration of large systemic doses of combined antibiotics, which are associated with unwanted side effects and poor compliance (Schreiber et al, 1999). Pharmaceutical aerosols, 1-5 μm once deposited may be removed by macrophage action before the dose is delivered, thereby reducing the bioavailability of the drug. Whereas for an antitubercular compounds it is the target region, as the pulmonary Mycobacterium tuberculosis infection is characterized by AMs containing large numbers of bacilli. Targeting the drug to AMs would be a rational addition to current therapy, potentially enhancing efficacy and reducing toxicity. Thus, a drug delivery system (Gupta and Hickey, 1991) targeted to the AMs might be effective, but has yet to be evaluated by direct administration to the lungs.

1.1 RESEARCH ENVISAGED

The project focuses on the pharmaceutical development of liposomal dry powder inhaler drug formulations of selected drugs; in vitro evaluation, optimization of flow and dispersion (deaggregation) characteristics of the formulations under development and the evaluation of the selected formulations in animals.

The proposed plan of work includes:

I. Literature reviews covering various aspects of liposomes in pulmonary drug delivery, dry powder inhalation formulation development and drug profiles of selected drugs like Isoniazid (INH) and Rifampicin (RFP).

II. To find an ideal liposomal form as far as encapsulation efficiency is concern. Liposomes containing drugs will be prepared by lipid film hydration method and reverse phase evaporation. The prepared liposomes will be characterized with respect to: Encapsulation efficiency, size and size distribution, lamellarity and trapped volume.

III. To incorporate the liposomal drug into an appropriate cryoprotectant to lyophilize and stabilize the formulation and developing formulations using different lactose grades or size to achieve desired fine particle fraction.

IV. Evaluation of the prepared Liposomal DPI will be carried out in terms of flow and dispersion properties using appropriate derived properties including, angle of repose, bulk density, compressibility and dispersibility.

V. Various parameters influencing DPI of selected drugs will be evaluated and optimized to establish control parameters at the end of studies.

VI. Stability studies of potential formulations with respect to potency, particle size, fine particle fraction and the physical changes like caking and discoloration.

VII. Comparative evaluation of the optimized formulations will be conducted for *In vitro* drug release and *In vivo drug absorption.*

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