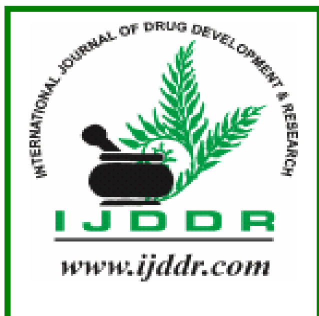

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Original Research Manuscript

LIPOSOMAL DRUG DELIVERY SYSTEM FOR ZIDOVUDINE: DESIGN AND CHARACTERIZATION

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ABSTRACT

Zidovudine is an antiretroviral drug with activity against Human Immunodeficiency Virus (HIV) Type 1. However, only temporary and limited benefits are observed in HIV infected patients treated with zidovudine alone or in combination to decrease viral burden, the rapid development of resistance have limited their long term efficacy. Hence, in the present work an attempt is being made to provide for stable drug delivery system with or having improved therapeutic index for zidovudine in form of lyophilized liposomes. Liposomes have been loaded by zidovudine (AZT) as a model drug using thin film hydration technique for targeted delivery of this drug. Four different formulations were prepared with various concentrations of egg phosphatidylcholine (EPC) and dipalmitoyl phosphatidylcholine (DPPC). A series of tests have been carried out to characterize the carrier vesicles in vitro, including loading parameters, drug release kinetics, particle size distribution, SEM analysis. Liposomes having acceptable loading parameters, released their drug content according to zero-order kinetics were selected for in vivo tissue distribution study. The AZT-loaded liposomes showed preferential drug targeting to liver followed by lungs, kidney and spleen. Totally, AZT-loaded liposomes seem to be a promising delivery system for targeting the drug to reticuloendothelial system (RES).

Keywords: *Zidovudine, liposomes, egg phosphatidylcholine, thin film hydration, dipalmitoyl phosphatidylcholine, human immunodeficiency virus.*

1. Introduction

Human Immunodeficiency Virus (HIV) is a retrovirus that causes irreversible destruction of immune system, leading to occurrence of opportunistic infection and malignancy. During last decade, even though attempts were being made to eradicate HIV, it was found that eradication of HIV is highly unlikely; an effective antiretroviral therapy is required on long term basis to maintain viral suppression and reduce disease progression. During this decade, effective therapies aimed at continued suppression of HIV replication and targeted at resting HIV reservoirs will be critical to prolong survivals and renewing hopes for cure [1, 2].

Zidovudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). However, only temporary and limited benefits are observed in HIV infected patients treated with zidovudine alone or in combination. The limited ability of this agent to decrease viral burden, the rapid development of resistance and toxic side effects have limited their long term efficacy. One major problem associated with the administration of zidovudine to patients is its poor ability to penetrate and target infected cells. Rapid drug clearance and toxicity like bone marrow depression are also some

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of the major drawbacks of zidovudine, which limits the use of it for antiretroviral therapy [3].

Amongst all carrier utilized for target oriented drug delivery, vesicular drug delivery system in the form of liposome is most extensively investigated. Liposomes are gaining popularity because of their inert biological nature, freedom from antigenic, pyrogenic or allergic reactions and similarity of primary components of liposomes with natural membrane. Liposomes are micro particulate or colloidal carriers which form spontaneously when certain lipids are hydrated in aqueous media [4, 5].

The macrophages play a central role in HIV pathogenesis, acting as reservoirs for dissemination of virus throughout the immune system. In early stage of the infection and throughout the clinically latent stage, HIV accumulates and replicates actively in lymphoid organs despite a minimal viral activity in peripheral blood. The uses of liposomes as a drug carrier are particularly relevant to control the progression of HIV disease. Since they are naturally taken up by reticuloendothelial cells, liposomes based therapy should concentrate the antiretroviral agents within cells susceptible to HIV infections and at the same time reduce the quantity of drug at the sites where it might be potentially toxic. Liposomes encapsulated drugs could therefore represent a convenient strategy to dissemination of HIV to the lymphoid tissues and protect the infected host from developing the characteristic immunodeficiency state [6, 7].

In the present study the zidovudine loaded liposomes were formulated by thin film hydration method. Four different formulations were prepared by using various concentrations of egg phosphatidylcholine and dipalmitoyl phosphatidylcholine. The prepared liposomes were subjected of characterization like % practical yield, % entrapment efficiency, vesicle size and surface morphology, surface charge, in-vitro drug release study, in vivo study and stability studies.

2. Materials and Methods

2.1 Materials

Zidovudine was received from Aurobindo Pharmaceuticals, Hyderabad India; egg phosphatidylcholine and dipalmitoyl phosphatidylcholine were kindly gifted by Lipoid GmbH, Ludwigshafen Germany; disodium hydrogen phosphate, potassium dihydrogen phosphate, sucrose and chloroform were from S.D. Fine-Chem Ltd., Mumbai India; all other chemicals were of the best quality commercially available.

2.2 Methods

2.2.1 Preparation of AZT loaded liposomes by thin film hydration Technique

Liposomes were prepared from various concentrations of egg phosphatidyl choline, dipalmitoyl phosphatidyl choline, cholesterol and drug. The conventional Bangham technique was used for the preparation of liposomes in research laboratory. In this method the lipids were casted as stacks of thin film from their organic solution using flash rotary evaporator (Buchi, Japan) under reduced pressure. The thin film of lipids was hydrated with aqueous buffer containing zidovudine at a temperature above the transition temperature of lipids. The heterogeneous population of multi lamellar vesicles, which were sonicated by bath sonicator (Remi Equipments , Bangalore) to produce small and more uniform sized population of liposomes. The liposomal formulations were dispensed in glass containers rapidly frozen at -40ºC, freeze-dried (Heto freeze-dryer, UK) for approximately 14 hrs under vacuum [8-10].

2.2.2 The content of drug in the extra vesicular medium

The content of zidovudine in extra vesicular medium after incubation of drug pretreated liposomes was determined by UV spectrophotometrically at 265 nm (Shimadzu UV/Vis spectrophotometer, 1201 Japan) using phosphate-buffered saline as blank [11, 12].

2.2.3 Scanning Electron Microscopy (SEM)

AZT loaded liposomes were prepared for scanning electron microscopy (SEM). Gold coating was performed by sputter coater (Polaron SEM coating system). Finally, cell samples were observed under the scanning electron microscopy (JSMT330A, JEOL) at a beam voltage of 15 kV [13, 14].

2.2.4 Vesicle size and size distribution

The vesicle mean diameter and size distribution were determined using particle size analyzer (Nanotrac, Japan). The suspension of liposomes was prepared from each batch, which was placed in sample holder. Then availability of number of particles was checked to ensure sufficient number of particles for detection. Then system was run for particle size analysis [13, 14].

2.2.5 Surface Charge

The surface charge on the vesicles produced a difference in the electric potential in millivolts between the surface of each particle and bulk of the suspending liquid was measured by using zeta meter (Zeta Meter 3+, USA) [10, 15].

2.2.6 in vitro drug release studies

The in vitro release of AZT from the liposomal formulation was determined using dialysis cassettes (Slide-A-Lyzer®3.5K, Pierce, U.S.A). Briefly, 100 mg of lyophilized product containing known amount of drug were suspended in phosphate buffer saline, which was placed in a dialysis cassettes (previously soaked in distilled water for 24 hrs). It was immersed in 50 ml of PBS (pH 7.4), maintained at 37 ± 1°C and stirred with the help of a magnetic stirrer. Aliquots were withdrawn at specific time intervals 1, 2, 4, 6, 8, 10 and 24 hrs from the receptor compartment and the sample was replaced with fresh PBS (pH 7.4) to maintain sink condition (Kosasih et al., 2000). The absorbance of the samples was measured at 265 nm after suitable dilution if necessary, using appropriate blank [16, 17].

2.2.7 in vivo tissue distribution studies

This study was carried out after obtaining the due permission for conduction of experiments from relevant ethics committee (K. L. E. S's College of Pharmacy, Belgaum) which is registered for "Teaching and Research on Animals" by committee for the purpose of control and supervision of experiments on animal, Chennai (Registration number 221/CPCSEA). This study was carried out to compare the targeting efficiency of drug loaded liposomes with that of free drug in terms of percentage increase in targeting to various organs of reticuloendothelial system like liver, lungs, spleen and kidneys. Experiments were performed on rats of 200 ± 20 gm weight.

Dose of zidovudine to be administered to rats was calculated according to body surface area ratio of rats to human being. Nine healthy adult rats weighing 200 ± 20 gm were selected. The rats were kept on a 12 hour light-dark schedule and they were fasted for 12 hrs. The animals were divided into 3 groups, each containing 3 rats. Group I received liposomes equivalent to 600 mcg/kg of zidovudine i.v. after redispersing them in sterile phosphate buffer saline solution. Liposomes from batch F2 were selected for the study. Group-II rats received 600 mcg/kg of pure zidovudine intravenously. Group-III rats were treated as solvent control and

were injected intravenously with sterile phosphate buffer saline solution.

After 8 hrs, the rats were sacrificed and their liver, lungs, spleen and kidneys were isolated. The individual organs of each rat were homogenized separately by using a tissue homogenizer with 5 ml PBS (pH 7.4) and the homogenate was centrifuged at 900×g for 30 min. The supernatant was collected and filtered through 0.2 μ filters (Minisart, Germany.) and analyzed spectrophotometrically after suitable dilution with phosphate buffer saline at 265 nm[16, 18- 20]

3. Results and Discussion

After incubation of liposomes with different concentrations of EPC and DPPC, the AZT was estimated in the supernatants by using spectrophotometric method,

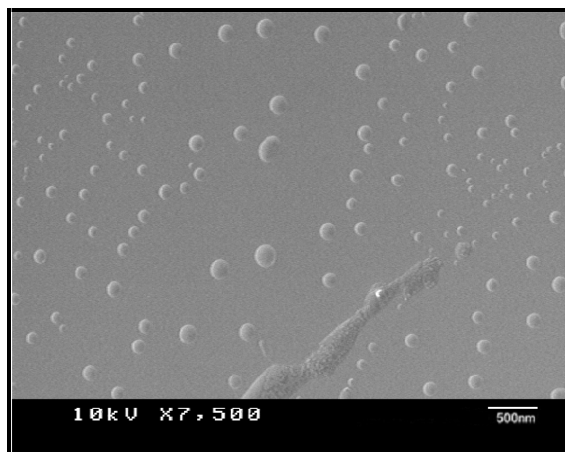

Fig-1. SEM image of (A) EPC- AZT liposome

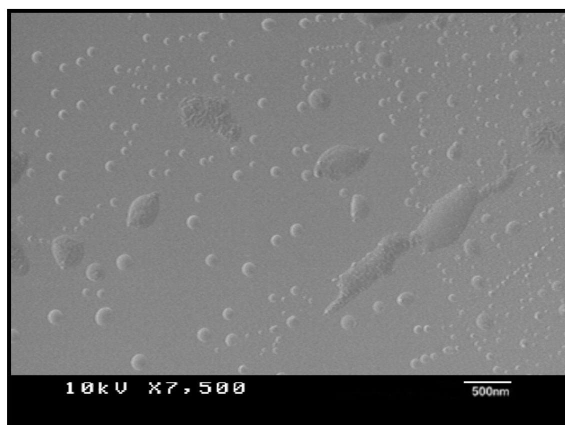

Fig-1. SEM image of (B) DPPC -AZT liposomes

Analysis of the obtained data indicates that liposomes incubated with drug linked only 47.59±0.356% to 56.04±0.887% of AZT. Relatively moderate entrapment of zidovudine in the liposomes could be ascribed to the hydrophilic nature of the drug, since encapsulation was depends upon lipophilicity of the cell membrane.

Due to the hydrophilic nature of zidovudine, it probably not gets intercalated preferentially in the lipid layer of the cell membrane. It was observed that drug entrapment efficiency increased with increase in concentration of phospholipids. To investigate the possible morphological changes of liposomes on loading process, samples of AZT liposomes were observed under the scanning electron microscope. SEM has been used to determine particle size distribution and to examine the morphology of fractured surface. **(Fig-1)** (A, and B) shows the images from scanning electron microscopy at magnification of x7500. As illustrated, the loading process with drug and different phospholipids resulted in the formation of spherical liposomes with very disperse sizes.

The average vesicle size was found to be 231.4 nm, 259.2 nm, 256.9 nm and 164.1 nm for formulation F1, F2, F3 and F4 respectively. The possible effects of these changes may affect the *in vivo* life span of the natural drug delivery system. Zeta potential of all formulated liposomes was found to be +5.56±0.568 mV, +9.89±0.468 mV, +6.47±0.976 mV and +8.36±0.713 mV for formulation F1, F2, F3 and F4 respectively.

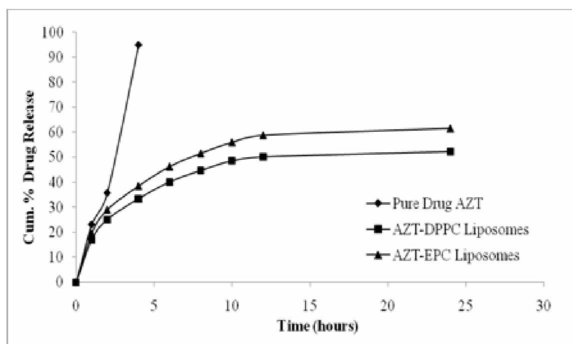

Fig -2. *in vitro* release profile of pure drug AZT and AZT liposomes formulated by using with two different Phospholipids. Data represents the mean ± S.E. (n=3).
Zidovudine (♦ - ♦), Zidovudine-DPPC liposomes (■ - ■), zidovudine-EPC (▲-▲)

The release profile of an entrapped drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behavior. The two formulations of AZT liposomes (EPC and DPPC) and pure drug AZT were subjected to *in vitro* release studies. These studies were carried out using dialysis cassettes (Slide-A-Lyzer®3.5K, Pierce, U.S.A) in phosphate buffer saline pH 7.4. The release data obtained for both formulations and pure drug were shown in **(Fig- 2)**, shows the plots of cumulative percent drug release as a function of time for different formulations and for pure drug. Cumulative percent drug release of pure drug was found to be 94.82% at 4 hrs, where as, cumulative percent drug release for EPC drug loaded liposomes were 61.42% and DPPC were 52.26% at 24 hrs. It was observed that drug release from the formulation containing (EPC) increased as compared to formulation treated with DPPC. It also indicates that, when compared with pure drug, the *in vitro* release of AZT from liposomes is prolonged over a period of 20 hrs. The *in vitro* release of both batches of liposomes showed an interesting diffusion controlled release throughout the study period, ensure the better entrapment of drug within the liposomes.

in vivo drug targeting studies were carried out for liposomes containing DPPC and with optimal particle size, high entrapment efficiency and satisfactory *in vitro* release. The comparison between the amount of drug targeted from liposomes and free drug in various organs is presented in **(Fig-3)**. The average targeting efficiency of drug loaded liposomes was found to be 35.87% of the injected dose in liver, 21.59% in lungs, 12.52% in kidney and 14.99% in spleen whereas accumulation of pure drug in liver was 23.91%, in lungs it was 13.52%, in kidney it was 09.26% and spleen 10.05±1.843% of the injected dose. These results revealed that, the drug loaded liposomes showed preferential drug targeting to liver followed by lungs, spleen and kidney. It was also revealed that, as compared to pure drug, higher concentration of drug was targeted to the organs like liver and lungs after administering the dose in the form of liposomes, higher drug targeting in liver, as compared to lungs, kidney and spleen may be attributed to uptake of the drug loaded liposomes by reticuloendothelial system and large size of liver as compared to other organs.

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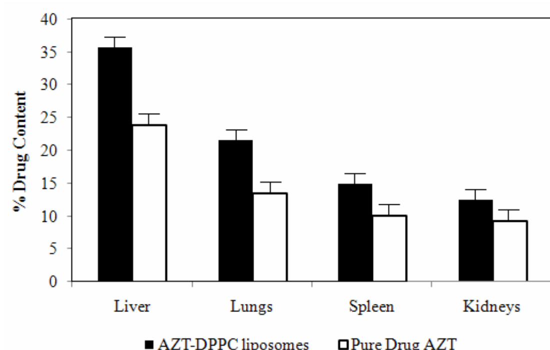

Fig-3. Comparison between the amounts of drug distributed from pure zidovudine and AZT-DPPC liposomes following i.v. administration. Data represents the mean ± S.E. (n=3).
AZT-DPPC liposomes (■), Pure drug AZT (□).

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