



PLAN AND SCOPE

OF THE THESIS



2.1 SCOPE OF THE THESIS:

Despite the toxicity problem of amphotericinB, it still remains a drug of choice for the treatment of many locally invasive and disseminated mycoses (Lee, et al., 1994).

Current research efforts are directed to improve the therapeutic index of amphotericinB largely focusing by incorporating amphotericinB into a lipid-drug delivery vehicle such as liposome or lipid complex. The advantage to such an approach is the ability to control the disposition of amphotericinB by altering its tissue distribution.

This would limit toxicity by targeting drug delivery more specifically to the sites of infection (Rapp, et al., 1997). Major drawback associated with the use of earlier, or conventional liposomal formulation is the tendency of liposomes to 'Leak' drug, while in circulation, the extensive uptake of these liposomes by tissues of reticuloendothelial system (RES) and the inability of liposomes to extravasate into infected tissue (Gabizone, et al., 1997).

At present, we can only speculate on the clinical significance of low serum levels of ABCL (Amphotericin-B Lipid Complex) and Amphocil (Amphotericin-B Colloidal dispersion) on the one hand, and the relatively high serum levels of AmBisome (Liposomal amphotericin-B) on the other (Khoo,

et.al.,1994). In the management of fulminant infections, we do not have adequate evidence to recommend dosages of lipid formulations equivalent to fungizone (DeMarry, et.al.,1994). Furthermore, data relating to the stability of such formulations are conflicting (Kirsch, et.al.,1988; Washington,et.al.,1993). Several strategic approaches have been adopted to circumvent, the stability related problems of the liposomal formulations both in vitro and in-vivo achieving with varying degree of success (Konsberg, et.al.,1990). All the same till today, none of these strategies have accepted completely. Hence, there is an urgent need to develop a simple and rational technique for preparation of stable and therapeutically effective liposomes.

Recently, a new method for the preparation of multilamellar liposomes was developed which avoids the usage of pharmaceutically nonaccepted solvents (Perrette, et.al.,1991). The method involves preparation of an initial proliposome, which is converted in-situ into liposome dispersion by a simple dilution step.

The recent patent application (Gregoriadis, et.al.,1995) describes a liposomal delivery system which involves cyclodextrin (CD) inclusion complexes It is observed that hydrophobic substances tend to leak out of liposomes which can be easily prevented by complexing them with CD's.

The developed chemically modified β -cyclodextrin are shown to be free from solubility as well as degradation problems generally associated with natural cyclodextrin (Masson, et.al.,1998).

Therefore, in the present investigation the first objective was to develop a novel hydroalcoholic method for preparation of liposome derived from proliposome containing amphotericin-B (AmB) complexed with different chemically modified β -cyclodextrin (β -CD's) with a view to reduce toxicity and to enhance therapeutic efficacy of AmB in-vivo. The method involves initial preparation of inclusion complex (AmB- β CDs) intercalated proliposome which is subsequently converted into a liposome dispersion by single dilution method. The AmB-liposome derived from proliposome exhibited superior entrapment stability as compared to liposomes prepared by employing conventional solvent-based techniques.

It has been demonstrated that lipid based formulation of amphotericinB like ABLC, ABCD and AmBisome have significantly reduced toxicity without much loss of antifungal activity (Clark, et.al.,1991 ;Clemons and Stevens, 1991; Proffit, et.al., 1991). All these formulations have altered pharmacokinetics and tissue distribution compared to fungizone (Clark, et.al.,1991; Olsen, et .al., 1991; Proffit, et.al. ,1991; Fielding, et.al., 1991, 1992; Janoff, et.al., 1993;

Lee,et.al.,1994). In order to ascertain as well as examine, the experiments were designed and also studied to find out whether reduction in toxicity and improvement of therapeutic efficacy is due to altered pharmacokinetics and tissue distribution of amphotericinB when delivered through liposomal AmB vesicles derived from proliposomes, its biodistribution in infected as well as normal mice was also studied.

Furthermore, the influences of varying lipid dose on the concentration in serum and tissue distribution of amphotericin B and different liposomal amphotericin B in treated rats were compared.

2.2 PLAN OF THE THESIS:

The work carried out has been summarized below: -

1. Preparation of liposomes :
 - 1.1. Preparation of sterically stabilized liposomally AmB derived from proliposome by hydroalcoholic method.
 - 1.1.1 Formulation of inclusion complexes of AmB with different chemically modified β - cyclodextrin.
 - a. Inclusion complex of AmB with hydroxy propyl β - cyclodextrin (AmB – HPBCD).

- b. Inclusion complex of AmB with sulpho butyl β - cyclodextrin (AmB – SBEB CD).

1.1.2 Entrapment of inclusion complex into liposomes :

- a. Entrapment of inclusion complex (AmB – HPBCD) into EPC liposomes.
- b. Entrapment of inclusion complex (AmB – SBEB CD) into EPC liposomes.
- c. Entrapment of inclusion complex (AmB – HPBCD) into DOPC liposomes.
- d. Entrapment of inclusion complex (AmB – SBEB CD) into DOPC liposomes.

2. Evaluation of proliposome based liposomal vesicles(PBLV)

2.1 Intercalation efficiency of PBLV.

2.2 Entrapment efficiency of PBLV.

2.3 Structural organization of proliposome mixtures.

2.4 ^{31}P -NMR measurements.

2.5 Particle size analysis.

2.6 Transmission electron microphotography.

2.7 Partition coefficient measurements.

2.8 In- vitro drug release studies.

2.9 In – vitro drug leaching studies.

2.10 Product shelf-life stability.

2.11 Toxicity Studies:

2.11.1 Determination of in – vitro toxicity of free amphotericin B and after intercalation in proliposome based liposomal vesicles.

2.11.2. Determination of in – vitro toxicity of free amphotericin B and intercalation in proliposome based liposomal vesicles.

2.11.2.A. Nephrotoxicity of free amphotericin B and PBLV entrapped inclusion complex.

2.11.2.B. Determination of LD₅₀.

2.12. Evaluation of therapeutic efficacy :

Comparative studies on the therapeutic efficacy of various liposome preparation of AmB on the aspergillosis mice model.

2.13. In – vitro tissue distribution studies in normal and infected mice:

2.13.1. Tissue distribution of AmB – incorporated in different liposomes in mice.

2.13.2. HPLC analysis of AmB in liposomes & in various organs.

2.13.3. Recovery of AmB in various tissues.

2.14. Influence of intralipid infusion on plasma pharmacokinetics.

2.15 Statistical analysis.