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Liposomal amphotericin B dry powder inhaler: Effect of fines on *in vitro* performance

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Received April 2, 2004, accepted May 13, 2004

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Pharmazie 59: 812–813 (2004)

The aim of the present investigation was to improve *in vitro* pulmonary deposition of amphotericin B (AMB) liposomal dry powder inhaler (LDPI) formulations. Liposomes with negative (AMB1) and positive (AMB2) charge were prepared by the reverse phase evaporation (REV) technique, extruded to reduce size, separated from unentrapped drug and lyophilized using an optimized cryoprotectant to achieve maximum drug retention. Lactose carrier (Sorbolac 400) in varying mass ratio with or without addition of fines (500# sieved Pharmatose 325M) in different mixing sequence were used to formulate AMB LDPI formulations. *In vitro* evaluation was done with twin stage impinger (TSI) for fine particle fraction. The lactose carrier containing 10% fines was found to be optimum blend at 1 : 6 mass ratio of liposome : lactose. The addition of fines and order of mixing fines were found to influence the fine particle fraction (FPF) significantly. FPF of LDPI formulations using a Rotahaler (Cipla, India) as delivery device at 30, 60 and 90 L/min were found to be 23.1 ± 1.5 percent and 17.3 ± 2.2 percent; 25.3 ± 1.8 percent and 19.6 ± 1.5 percent and 28.4 ± 2.1 percent and 22.9 ± 1.9 percent for AMB1 and AMB2 respectively.

Improving the drug delivery to the lungs from a DPI formulation can be made possible by various techniques like smoothing the carrier surface (Ganderton, 1992), reducing the particle size of the carrier (Steckel et al 1997) and use of a ternary powder mix formulation (Staniforth 1996 a). Addition of micronized lactose to coarse lactose carrier was found to improve the dispersion and deaggregation of salbutamol sulphate and spray dried bovine serum albumin (Lucas et al 1998). Also, techniques like spray drying the drug with phospholipid composites in a suitable range for pulmonary delivery (Kim et al 2001) or the dissolution of lecithin in chlorofluorohydrocarbon and the formation of liposomes *in situ* (Farr et al 1987) or nebulization of the preformed liposomes (McCallion et al 1996) can be attempted for liposomal drug delivery to lungs. We have studied the delivery of liposomal ketotifen and liposomal budesonide DPI by blending the lactose carrier with preformed liposomes as described previously and found the fine particle fraction (FPF) not more than 21% (Joshi et al. 2001 a, 2001 b). The aim of the present investigation was to study the effects of addition of fines and the addition sequence of fine carrier on *in vitro* deposition of the formulations using TSI at different flow rates.

Multilamellar vesicles (MLVs) composed of drug (5mg), HSPC, cholesterol, α-tocopherol (1% of PC) and either soyaphosphatidylglycerol (SPG-3) (AMB1) or stearylamine (AMB2) of AMB were prepared by the modified reverse phase evaporation technique (Cortesi et al. 1999) by using 0.01M Tris buffer pH 6.5 containing 1mM EDTA (ratio of aqueous phase : organic phase was 1 : 5) with intermittent vortexing. The formed liposomal dispersion was extruded through a 2 μm polycarbonate membrane above the phase transition temperature and separated from unentrapped drug by controlled centrifugation. To achieve high PDR, lyophilization was carried out for 48 h using sucrose as cryoprotectant in a mass ratio of 1 : 5.

To prepare LDPI formulations, the porous cake of liposomes obtained after lyophilization was sieved (200# and 240#) and filled in capsule size "2" containing 250 μg of AMB. Similarly the sieved lyophilized powder was mixed with Sorbolac 400 in different mass ratio (1 : 2 to 1 : 8). The addition of fines in the range of 5%–15% and addition sequence of fines were investigated i.e., first fines were mixed with carrier and then mixed with lyophilized liposomes or fines were mixed with lyophilized liposomes and then mixed with the carrier (Table 1).

The volume of capturing solvent (methanol) in the upper (stage 1) and lower (stage 2) were 7 and 30 ml respectively in TSI (B. P. Apparatus A) (British Pharmacopoeia 1993). Rotahaler (Cipla, India) was used as delivery device at flow rates of 30 ± 2 L/min, 60 ± 2 L/min and 90 ± 2 L/min for 5 s for 5 capsules. The inhaler body, capsule shells, mouthpiece, stage 1 and stage 2 were washed five times with methanol and analyzed to measure the amount of drug retained as described before (Ruijgrok et al. 2000). The fine particle dose (FPD) was denoted as the quantity (μg) of the particles per capsule that deposited in the lower stage of the TSI after aerosolization at 30 L/min, 60 L/min and 90 L/min. Each capsule contained a powder mass of 84.8 ± 2 mg (for AMB1) and 71.0 ± 2 mg (for AMB2)

Table 1: Optimization of LDPI formulation

Variable studied	Percentage FPF for AMB1[a]	Percentage FPF for AMB2[a]
Effect of liposome: lactose ratio (Sorbolac 400)		
1 : 2	12.3 ± 2.2	8.5 ± 2.4
1 : 4	15.1 ± 3.0	11.6 ± 2.2
1 : 6	17.5 ± 2.4	13.2 ± 3.1
1 : 8	16.4 ± 2.7	11.9 ± 2.8
Effect of percentage of carrier (liposome: lactose ratio was 1 : 6)		
5%	19.2 ± 2.5	14.9 ± 2.5
10%	22.5 ± 2.2	16.8 ± 2.2
15%	20.1 ± 1.9	14.6 ± 2.3
Effect of sequence of addition of fines (10%, 500# sieved Pharmatose 325M)		
Fines + carrier + lyophilized liposomes	25.3 ± 1.8	19.6 ± 1.5
Fines + lyophilized liposomes + carrier	22.1 ± 1.6	18.1 ± 1.9

[a] n = 5 (± SEM)

Table 2: Comparative characterization of potential batches of AMB LDPI formulations

Parameters	AMB1						AMB2					
	30 L/min		60 L/min		90 L/min		30 L/min		60 L/min		90 L/min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FPD (μg)	56.6	1.8	64.5	1.5	73.9	2.0	40.7	1.7	47.0	1.4	56.1	1.8
FPF (%)	23.1	1.5	25.3	1.8	28.4	2.1	17.3	2.2	19.6	1.5	22.9	1.9
Dispersibility (%)	26.1	1.5	28.4	2.2	31.8	2.1	20.8	2.4	23.4	1.8	27.1	1.3
Effective Index	45.2	1.2	47.5	1.9	50.4	1.4	37.9	2.0	40.5	1.6	44.0	1.7

Control: Asthalin (Cipla Ltd., India):
Delivery device: Rotahaler (Cipla Ltd., India)
FPF = 27.1 ± 2.0, EI = 48.6 ± 1.7 at 60 L/min

equivalent to nominal dose of 250 ± 7 μg AMB. The recovered dose (RD) was taken as the total quantity of drug recovered per capsule after each actuation, while the emitted dose (ED) was that emitted from the inhaler device. Percent emission was calculated as the percentage of emitted dose to total dose. FPF was the ratio of FPD to RD, while dispersibility was the percentage of FPD to ED (Table 2). As a control, a marketed preparation (Asthalin Rotacaps, Cipla Ltd., India) containing salbutamol sulphate powder was used and the FPF determined at 30 L/min, 60 L/min and 90 L/min flow rate using Rotahaler as the delivery device (Table 2). The Rotahaler device was rinsed with methanol to determine the device fraction and Effective Index (EI) (Hino et al. 1998).

Effective index is the geometric mean of the total emitted dose and FPF, represented by the equation (Hino et al. 1998):

$$EI = \sqrt{(100 - DF) \times FPF} \qquad (1)$$

where, DF is the device fraction.

Significant differences were calculated by ANOVA and mutual differences were detected with Students t-test and differences at $P < 0.05$ were considered as significant.

The liposomes of AMB were prepared by REV technique using ethyl acetate and ethanol (1 : 1) as organic solvents and 10 mM Tris buffer pH 6.5 containing 1 mM EDTA as aqueous phase. Liposomes were extruded to reduce size, separated from unentrapped drug and lyophilized using optimized cryoprotectant to achieve maximum percent drug retention. Maximum PDE estimated in AMB1 and AMB2 liposomes were 95.8 ± 1.5 and 87.9 ± 1.3 respectively. The optimum lipid: sucrose mass ratio was found to be optimum at 1 : 5 with PDR of 96.6 ± 1.8 and 94.7 ± 2.4 for AMB1 and AMB2 respectively.

The mass ratio of liposomes : Sorbolac 400 at 1 : 6 resulted in FPF of 17.5 ± 2.4 and 13.2 ± 3.1 percent for AMB1 and AMB2 respectively. Optimum concentration of carrier is required to achieve detachment of liposomal drug from carrier molecule. Carrier concentration is less or more than optimum resulted in too low FPF or no further increase in FPF. Further the effect of increasing fines from 5% to 10% resulted in higher FPF of 22.5 ± 2.2 and 16.8 ± 2.2 percent respectively. Furthermore the addition sequence of fines such as fines first mixed with carrier and then mixed with lyophilized liposomes resulted in FPF of 25.3 ± 1.8 and 19.6 ± 1.5% with significantly different EI. High-energy adhesion sites (HA) of lactose bind strongly to the liposomal drug particles and low-energy adhesion sites (LA) allow the formation of more reversible bonds with liposomal drug. This results in efficient detachment of liposomal drug from the carrier as observed with plain DPI formulations (Staniforth 1996 b). Hence, 10% sieved Pharmatose 325 M added to LDPI formulation occupies HA sites leaving LA sites for attachment of liposomal drug and thus resulted in higher FPF. Based on EI, the deposition of liposomal AMB was more efficient in case of AMB1 than the AMB2. The FPF ratios of control to that of the developed LDPI formulations were 0.93 and 0.72 and EI ratios were 0.97 and 0.83 for AMB1 and AMB2 respectively. The EI of AMB1 was found to be better than the AMB2 suggestive of more effective liposomal drug deposition in to lung. It may be due to triboelectrification or charge generation in liposomal powder during dispersion via the Rotahaler. The lower ratio of EI/FPF is suggestive of efficient dispersion of AMB1 from the device but unlike the control more proportion of the dispersed powder has been deposited in the upper respiratory tract (Hino, 1998).

Acknowledgement: The authors wish to thank Council of Scientific and Industrial Research (CSIR, India) for providing financial assistance to carry out research in this field.

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