M1311

Development of the Liposomal Amphotericin B Release

Jie Tang[1,2], Frances M. Acevedo[1], Wenmin Yuan[1], Zhipeng Dai[3], Dan Li[1], Nan Zheng[4], Wenlei Jiang[4], Charles Noble[3], Mark Hayes[3], Francis C. Szoka[3, 5] and Anna Schwendeman[1]

[1]Department of Pharmaceutical Sciences and Biointerfaces Institute, University of Michigan; [2]School of Food and Bioengineering, Xihua University; [3]ZoneOne Pharma, Inc.; [4]Office of Generic Drugs, Food and Drug Administration; [5]Department of Bioengineering and Therapeutic Sciences, UCSF.

PURPOSE

The goal of this study is to develop a USP 4 apparatus flow-through assay for measuring Amphotericin B (AmpB) release from liposomal formulations capable of discriminating the alterations in liposomal production, compositions and physical characteristics.

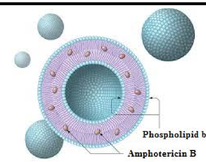

Fig.1 Schematic picture of AmBisome®

METHODS

The basic release media used in the assay contained 5% sucrose, 10 mM HEPES and 0.01% NaN_3 (pH=7.4). In the single-unit vial-based assay, AmBisome® or free Amphotericin B (Amp B) were placed in Float-A-Lyzer® dialysis tubes (Spectrum Labs, molecular weight cut-off 300 kDa) and inserted in release media, then incubated in a shaking water bath. To increase the *in vitro* release of AmBisome®, sodium dodecyl sulfate (SDS), isopropanol (IPA), hydroxypropyl-cyclodextrin (HP-CD) and γ-cyclodextrin (γ-CD) were used as solubilizer in the release medium. The effect of temperature, drug concentration and solubilizer concentration on the Amp B release were further investigated. The assay was adapted to USP 4 apparatus flow-through apparatus Sotax® CE7 Smart. Each cell contained 80 mL of the release media re-circulated at 16 mL/min flow rate. Then the method was validated by performing the same AmBisome® samples on three different days. The ability of USP-4 release assay to discriminate different commercial AmpB preparations was examined.

A B

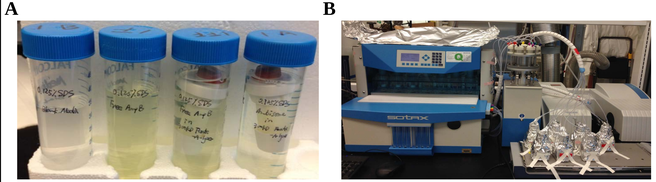

Fig. 2 Pictures of single-unit vial-based assay system (A) and USP 4 apparatus CE7-smart (SOTAX ®) dissolution system (B)

RESULTS

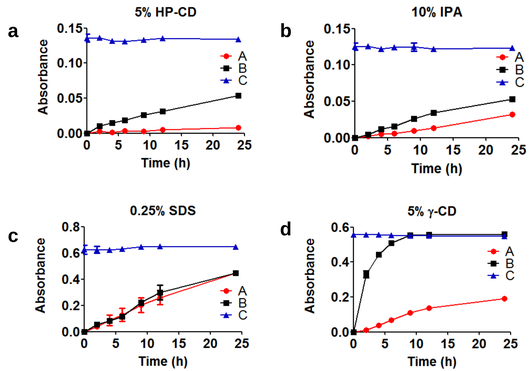

Fig. 3 Comparison of different solubilizes in release medium via the single-unit vial-based assay at 45 ℃, including 0.25% SDS (a) , 10% IPA (b), 5% HP-CD (c) or 5% γ-CD (d), A: AmBisome® in float-a-lyzer; B: Free AmpB in float-a-lyzer; C: Free AmpB in release medium. Final Amp B concentration was 10 μg/ml.

•When using 5% HP-CD or 10% IPA as solubilizer (a, b), obvious precipitations were observed in the release medium, therefore the absorbance of all the groups were relatively smaller.

• Addition of 0.25% SDS (c) resulted in efficient free AmpB dissolution (C), and faster release of Amp B (A, B). However, the size of AmBisome® decreased from 100 nm to 20 nm, indicating liposome structural degradation (data was not shown).

•When 5% γ-CD was added (d), no structural degradation of liposome or precipitation of AmpB was observed, but AmBisome® release was limited and required further improvement.

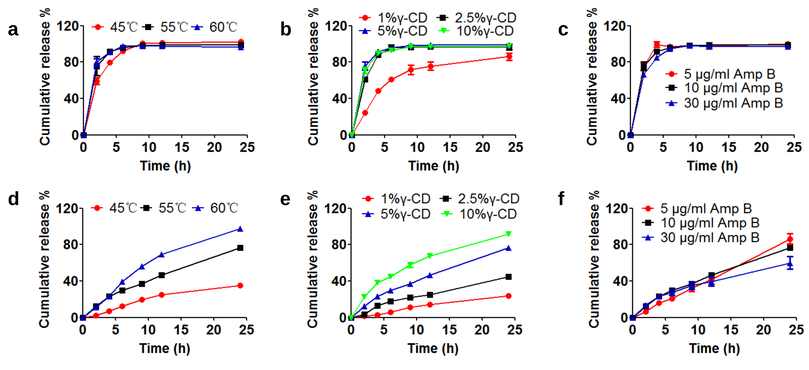

Fig. 4 Optimization of release condition for Amp B (a, b, c) and AmBisome® (d, e, f) by the single-unit vial-based assay, including effect of temperature with 10 μg/ml Amp B and 5% γ-CD (a, d), the effect of γ-CD concentration with 10 μg/ml Amp B at 55 ℃ (b, e) and the effect of Amp B concentration with 5% γ-CD at 55 ℃ (c, f).

According to the results of Fig. 4, 55 ℃, 5% γ-CD and10 μg/ml Amp B were chosen as final release condition. Under this condition, not only free Amp in float-a-lyzer released fast and thoroughly, the release of AmBisome® in float-a-lyzer could reach at 76%.

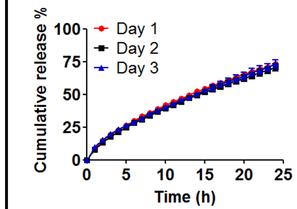

Fig. 5 Validation of the USP-4 release assay on Sotax®. The release experiments were performed using the same AmBisome® sample on three different days.

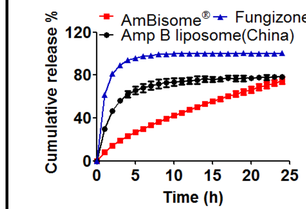

Fig. 6 The USP-4 release assay on Sotax® discriminates the Amp B release from different commercial Amp B formulations. The AmpB liposome was provided by Shanghai Asia Pioneer Pharmaceutical Co. Ltd.

CONCLUSIONS

Amp B could be efficiently released from AmBisome® (final Amp B concentration equals 10 μg/ml) in the medium containing 5% sucrose, 10 mM HEPES, 0.01% NaN_3 and 5% γ-CD (pH = 7.4) within 24 h at 55 ℃. The current USP 4 release method can be used to distinguish different Amp B products. It serves as a starting point for the development of an USP 4 apparatus flow-through assay which may be able to guide the design of generic products and provide an accurate predict *in vivo* drug release kinetics.

AKNOWLEDGEMENTS

This project was supported by FDA grant (1U01 FD005249-01).
Views expressed here do not necessarily reflect the official policies of the Department of Health and Human Services nor does it imply endorsement by the United States