**INTRODUCTION**

- Treprostinil (TRE) is a prostanoid analogue used to treat pulmonary arterial hypertension (PAH).
- In this study, we designed a TRE delivery system based on a lipid nanoparticle (LNP) component for the encapsulation and sustained release of drug.
- Uniform, mono-dispersed: liposomal nanoparticles (TRE-LNPs) (Figure 1) were composed of (i) TRE, (ii) a cationic lipid (DSPE-PEG2000) and (iii) a hydrophilic “slur” to stabilize the core of the particle, and (iv) a sodium concentration of 150mM to adjust the pH to the LNP nanoparticles.

**AIMS**

- To design an injectable TRE formulation for the treatment of PAH that would have an improved pharmacokinetics (PK) profile relative to the current injectable TRE therapy for PAH Tyvaso® to facilitate a once-daily dosing schedule.
- To achieve this, we developed a bioavailable TRE vehicle carrier with an optimized drug payload and particle size to support a sustained release response relative to free TRE.

**METHODS**

**TRE-LNP Production**

- Solvent flash precipitation via microliter flow dosing was used for the one-step, continuous-flow synthesis of uniform nanosized TRE-LNPs (Figure 2).
- In this process, a stream of alcohol-solvated TRE and lipid is impinged against aqueous streams positioned perpendicular to the TRE stream.
- As the aqueous streams meet and laterally focus the miscible-solvated lipid stream, the hydrophilic “slur” and lipids co-emulsify, producing a resultant suspension in which the lipids are increasingly less solvated.
- This causes the lipids to self-associate into intermediate aggregates that eventually close themselves into spherical nanosized LNPs.

**RESULTS**

- In vitro TRE-LNP Characterisation Methods
  - Release kinetics were studied by dialysis:
    - TRE concentration was measured using HPLC/MS/MS analysis.
    - Initial concentration of TRE was 100 µg/mL.
    - Dialysis cell was incubated at 37°C for 24 hours.
  - Efficiency released in a monolayer cell using an appropriate description of these methods, see post HER Chest (2020):
    - Tissue chamber (TC)-cell (TC-Cell) was transected in vitro with the philosophy of Zelka et al. (2018) and the membrane of TRE-LNPs.
    - Tissue chamber (TC) cells (TC-Cell) were transfected with the pCLE-15-fluorescence (FRET 2.0) and the membrane of TRE-LNPs.
  - In vitro bioavailability of TRE was measured using a one-step, continuous-flow synthesis of uniform nanosized TRE-LNPs.
  - In situ bioavailability of TRE was measured using an in vitro/in vivo release study.

**CONCLUSIONS**

- In the present study, we sought to develop an injectable TRE-LNP formulation for the treatment of PAH to improve the delivery of the therapeutic benefit and the solubility of TRE. Optimisation of TRE-LNP formulation was based on particle size and TRE release kinetics.
- Assay was done in vitro in CHO-K1 cells using a phospholipase assay and in vivo in an acute hypoxia rat model of PAH.
- A gradual increase in cAMP activation of CHO-K1 cells suggested a delayed-release profile of the TRE nanoparticle formulation relative to the free drug.
- In the in vivo model, the pulmonary vasodilatory activity of inhaled TRE-containing LNPs was extended beyond that of free TRE in solution, which is consistent with an extended PK profile of the drug observed in excised blood plasma.
-包装后的LNP纳米片的形成进一步延长了肺血管舒张作用的持续时间，其效果优于单次给药中使用的LNP纳米片。
- To further improve nanoparticle retention of TRE, we developed a derivate TRE-LNP made by covalent attachment of an alkyl chain (used by Lebherz et al. Insmed, Inc., Bridgewater, NJ, USA).
- We believe that this approach would result in a sustained vasodilatory response well beyond that observed with TRE-LNPs and free drug.

**ACKNOWLEDGEMENTS**


**REFERENCES**

[1] Aims, INTRODUCTION: METHODS – As the aqueous streams meet with and laterally focus the miscible-solvated lipid stream, the hydrophilic “slur” induces to stabilize the core of the particle, and (ii) a sodium concentration of 150mM to adjust the pH to the LNP nanoparticles.

[2] METHODS – TRE-LNP Production: Solvent flash precipitation via microliter flow dosing was used for the one-step, continuous-flow synthesis of uniform nanosized TRE-LNPs (Figure 2).

[3] RESULTS – In Vitro TRE-LNP Characterisation Methods: Release kinetics were studied by dialysis:

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