Dual Drug-Loaded Cubosome Nanoparticles for Pulmonary Tuberculosis Treatment

Authors: Alec M. Diorio, Parasharamulu Kommarajula, Suyash M. Patil, Nitesh K. Kunda
Presenting Author: Alec M. Diorio
Affiliation: Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Jamaica, NY 11439, USA
Corresponding Author's email: kundan@stjohns.edu

Purpose
Purpose: Tuberculosis (TB), caused by Mycobacterium tuberculosis (M.tb), is infectious and fatal. Current TB treatments include oral dosages of delamanid (DLD) and bedaquiline (BDQ) but suffer from low local bioavailability, resistance, and systemic side effects. Hence, we propose formulating DLD-BDQ co-loaded cubosomes (BDPC). Cubosomes are lipid nanocarriers that improve drug aqueous solubility, capable of nebulization, minimize systemic side effects and facilitate efficient uptake of drugs into macrophages wherein TB resides.

Methods
Methods: The minimum inhibitory concentration (MIC) in M.tb (H37Ra, ATCC 25177TM) was determined using resazurin microtiter assay. The dual-drug effect was determined using checkerboard assay and fractional inhibitory concentration index (FICI). Phytantriol cubosomes were prepared using single-emulsion solvent evaporation method and were characterized for particle size using Malvern Zetasizer Nano ZS. The solid-state characteristics were determined using differential scanning calorimetry. Further, the cellular internalization of coumarin 6-loaded cubosomes into macrophages (THP-1, ATCC TIB-202TM) was confirmed using microscopy.

Results
Results: The MIC for DLD and BDQ was 0.250µg/mL and 0.063µg/mL, individually and. 0.063µg/mL and 0.016µg/mL, in combination, respectively, showing a 4-fold decrease in both MIC values. Based on MIC values, the optimized drug ratio was determined to be 1:4 BDQ:DLD. Furthermore, the FICI calculated for the optimized ratio showed an additive/synergistic effect with a FICI of 0.5, allowing for a reduction in the effective concentration of antibiotics and overall systemic side effects. When formulating the combination therapy, initially adding 1:6 BDQ:DLD resulted in the optimized ratio (1:4) being encapsulated. The combination was successfully loaded into BDPC with an encapsulation efficiency of 71.79±2.8%w/w and 97.58±1.95%w/w for DLD and BDQ. Further, the BDPC showed an average particle size 193.5±6.5nm (PDI 0.17) and zeta potential -8.73±6.4mV. The encapsulation of the drugs was confirmed by the absence of melting peaks in DSC for each drug in the cubosomes thermogram. Macrophages showed rapid internalization (<2h) of cubosomes, can be attributed to small size, allowing them to undergo phagocytosis and release the drug.

Conclusion
Conclusion: DLD-BDQ combination therapy showed an additive/synergistic effect against M.tb and was successfully loaded at an optimized ratio into PHY cubosomes nanocarriers with a size less than 200nm and complete encapsulation of the therapy.

Keywords: Tuberculosis, Cubosome, Nanoparticle, Inhalation, Antibiotic