Development and Characterization of Emulsomes for Macrophage Targeted Multidrug Therapy against Tuberculosis

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ABSTRACT SUMMARY

Glyceryl tridecanoate (tricaprin) based nanosize lipid particles (emulsomes) stabilized by soya phosphatidylcholine (PC) were prepared as a new drug delivery system for alveolar macrophage targeting for the treatment of tuberculosis. Multidrugs (rifampicin, isoniazid, pyrazinamide) encapsulated emulsomes dry powder inhaler (DPI) resulted in high concentration of drugs in the lungs.

INTRODUCTION

Mycobacterium tuberculosis is an opportunistic pathogen based cellular infection specifically confined to phagosomes of macrophages. Though effective antitubercular drugs are available, the resistance towards these drugs is reported which is responsible for incomplete eradication i.e. responsible for residual infection prone for reoccurrence (reactivation). The pathogens largely involve G-glycoprotein efflux to pump out the drug. It is therefore, in practice accepted clinically that different drugs which act/work following different modes and mechanisms of action are effective in the treatment of tuberculosis (T.B.). However, this needs an excessively long period of treatment varying from 6-9 months with frequent regimen of dosing where most of the drugs are associated with contraindicative manifestations associated with their systemic levels.¹

It is therefore in bio-clinical terms desirable that by using available optional strategies, differentiated concentration should be produced selectively higher in cellular tropics of infection compared to blood plasma pool. This can be achieved using inhalable or aerosolized nano carriers delivered to macrophages through receptor mediated phagocytosis resulting into many folds higher intracellular concentration into the cellular tropics (i.e. phagosomes).²

EXPERIMENTAL METHODS

Multidrugs (rifampicin, isoniazid and pyrazinamide) encapsulated emulsomes were prepared by cast film technique followed by homogenization. Emulsomes were modified by coating them with macrophage-specific ligand (O-palmitoyl mannan, OPM). The surface modified emulsomes and their plain counterparts were characterised for size, shape, zeta potential, drug entrapment efficiency and in vitro drug release profile using particle size analyzer, scanning electron microscope, transmission electron microscope, sephadex G 50 mini column centrifugation and dialysis tubing method respectively. Drugs were analyzed using simultaneous HPLC estimation technique during characterization. The optimized lyophilized emulsome powder was mixed with lactose carrier in mass ratio of 1:5 to prepare dry powder for inhaler. Dry powders were further characterized for angle of repose (fixed funnel method), moisture content (Karl Fisher volumetric titration method), cascade impaction (cascade impactor) and morphology (scanning electron microscopy). The in-vivo organ distribution studies were performed in albino rats.

RESULTS AND DISCUSSION

Optimum PC:tricaprin w/w ratio was found to be 1:1 for emulsome formulation which could entrap maximum amount of drugs with acceptable particle size. Optimum sonication time was recorded to be 12 min, which gave particle size of 0.25±0.02 µm. After optimizing the process parameters, emulsomes were coated...
with macrophage specific ligand OPM. Optimum ratio of OPM:lipid was recorded to be 4:6 (w/w). Optimum incubation time was recorded to be 4 h. The TEM photograph suggested that the emulsomes are spherical in shape and multilamellar in nature (Figure).

Figure 1. TEM image of emulsomes (200000X)

Photomicrograph also reveals the solid state of lipid which is responsible for controlled pattern of drug release. The presence of coating on the emulsome surface could also be appreciated from the TEM, which indicates surface intervening and anchoring of mannose terminating ligand (Figure 2).

Fig 2: TEM image of OPM coated emulsomes (80 K X)

SEM analysis shows the distribution and the porous nature of DPI formulation as depicted in the figure 3.

Fig 3: SEM photograph (200X) of optimized emulsome DPI formulations

The in vitro drug release profile of various drugs from lyophilized plain and coated emulsome formulations and their respective DPIs was found to be sustained up to 48 hours with DPIs showing highest release. The organ distribution data suggest that the emulsomes DPI are not only effective in rapid attainment of high-drug concentrations in lungs but could also maintain the concentration over a prolonged period of time when compared against the free drugs DPI.

CONCLUSION
In conclusion, the proposed multi-drug emulsome-based DPI shows tremendous potential for alveolar macrophage targeting for the treatment of tuberculosis. Further clinical trials will be needed to confirm the efficacy of these systems.

REFERENCES

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