PLA microparticles for pulmonary delivery of AntiTB drugs: biodistribution study

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Introduction

Tuberculosis (TB) is a communicable infectious disease caused by *Mycobacterium tuberculosis* (MTB) which commonly affects the lungs. Current therapy against pulmonary TB mainly consists of oral administration of therapeutic drugs or antibiotics such as isoniazid (INH), pyrazinamide (PZA), ethambutol and rifampicin (RFP) which require long periods of high-dose daily drug administration to achieve complete cures (H. S Cox, et al 2008). To kill the bacilli residing in alveolar macrophages, it is essential to deliver a sufficient amount of drug into the lungs. Thus, the failure of TB patients to comply with drug therapy, the manifestation of significant side effects, and the need to target the drug therapy to the alveolar macrophages, emphasizes the inadequacy of current oral therapies for TB. To minimize toxicity and improve patient compliance, extensive efforts have been made to develop inhalation drug delivery systems to target the alveolar macrophages that harbor the TB bacteria (P. Muttil, et al 2007). In the case of pulmonary TB, delivering the drug directly to the site of infection as inhalation aerosols can also bypass first-pass metabolism and maintain local therapeutically effective concentration with decreased systemic side effects. Biodegradable microparticles can be used for targeted delivery of anti-TB drugs to MTB infected macrophages. In present study, a dry powder inhalable (DPI) microparticles comprising anti-tuberculosis drugs encapsulated in biodegradable polymers were developed for the treatment of pulmonary tuberculosis. Poly L-lactic acid (PLA) microparticles incorporating a high payload of rifabutin and isoniazid were fabricated by spray drying to develop sustained release delivery system for drug. Microparticles of desired high encapsulation efficiency and sustained release characteristics were produced having a diameter range of 1-10µm (Malvern Mastersizer 2000). The time course of tissue biodistribution following a single inhalation dose of microparticles was evaluated. We studied pharmacokinetic tissue distribution and compared with high dose of intravenous administration of free soluble rifabutin and isoniazid.

Materials and methods

Fabrication of drugs incorporated inhalable microparticles by spray drying

Microparticles were prepared by spray drying method. The solution of 5 parts of rifabutin prepared and 10 parts of PLA was prepared in dichloromethane at room temperature by mixing. The solution containing 5 parts of isoniazid in methanol was prepared by warming at 37^{o} C. The homogeneous solution prepared by mixing both solutions with constant stirring just spray drying. This solution was spray-dried through nozzle (0.7mm) at air flow rate of 600 NL/h; feed rate 5ml/min, feed concentration was 3% of total solid, inlet temperature 54±2^{o}C and outlet temperature 33±5^{o}C, using a Buchi 190 spray-dryer. Free-flowing microparticles were collected from the product collection chamber (P. Muttil, et al 2007).

Charcterization of microparticles

Particle size analysis: was determined using a laser-based analyzer (Mastersizer 2000, Malvern Instruments, UK) at laser obscuration factors ≥ 10%. ***Differential scanning calorimetry (DSC)***: Samples (2 mg) were weighed in aluminum pans, sealed and subjected to temperature ramping at 10°C/min from 10 to 300°C under nitrogen using Pyris Diamond DSC system (Perkin-Elmer). ***Electron microscopy:*** Samples was mounted on metal grids and analyzed at 30 KV voltage after gold coating.

Dosing, drug extraction and sample analysis (HPLC) in mice

***Dosing*:** Animal experiments were conducted with 120 BALB/c mice. Sixty-four mice were assigned to the Isoniazid Group. Of these, 32 received inhalations, while the remaining were administered intravenous injections of 100 µg isoniazid in PBS (sterile). Animals were administered dry-powder inhalation of microparticles using an in-house inhalation apparatus. Briefly, about 20 mg of microparticles were charged into the apparatus and fluidized by 30 actuations over 30 secs. ***Drug extraction*:** Animals were sacrificed at time-points of 10 min, and 6, 12, 24, 48, 72, and 96 h. Tissues of liver, lungs, and kidney were excised off and homogenized in PBS. Drugs were extracted from 500 µl of tissue homogenates. Isoniazid was extracted using chloroform: butanol (70:30v/v) by vortexing for 1 min followed by centrifugation. The aqueous layer was separated by centrifugation and 200µl aspirated and analyzed using HPLC. A solvent system comprising equal parts of dichloromethane and *n*-pentane was added to the samples, vortex-mixed for 1 min, and centrifuges. The organic layer was transferred to a glass test tube and vacuum-dried. Just prior to injection, the sample was reconstituted in 500µl of methanol. ***Analysis*:** Isoniazid was eluted with 3% acetonitrile in triethylamine acetate (TEAA) buffer at pH 6.0, at 1 ml/min with the detector set at 262 nm. The drug eluted at ~6 min. Acetonitrile and 0.05 M potassium dihydrogen phosphate, pH-4.10 (55:45 v/v) were used to elute rifabutin at 1ml/min with the detection wavelength at 275nm. Rifabutin was eluted at ~5.5 min.

3. Results and Discussion

Spray drying method yielded rifabutin and isoniazid encapsulated PLA microparticles having median particle diameter between 1-10µm with narrow size distribution which is appropriate for endocytosis by alveolar macrophages. The particle size distribution for microparticles is shown in Fig.1.

Fig 1: Particle size distribution of drugs incorporated microparticles

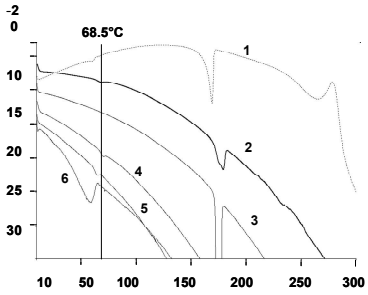

Figure 2: DSC thermograms melting temperature of PLA microparticle matrix (curve 2) containing isoniazid (curve 1), rifabutin (curve 6) and the combination of both (curve 5). Isoniazid (curve 3) & rifabutin (curve 4)

DSC was performed in order to investigate polymer and drug interaction. Glass-transition temperature of PLA microparticle matrix was about 68.5 °C. This was reduced drastically as a result of incorporation of rifabutin alone, less so when isoniazid alone was present in the matrix and to an intermediate value when both drugs were present in the matrix (fig-2). Surface morphology of drugs loaded microparticles by electron microscopy has shown in fig-3

Figure:3 Drug encapsulated Microparticles surface morphology by electron microscopy.

Figure4: Inhalation of PLA microparticles using in-house apparatus

In present study, we showed that inhalation administration of rifabutin and isoniazid drug resulted in effective accumulation in lungs. However, administration of intravenous administration do not provide selective accumulation in targeted organ i.e. lungs.

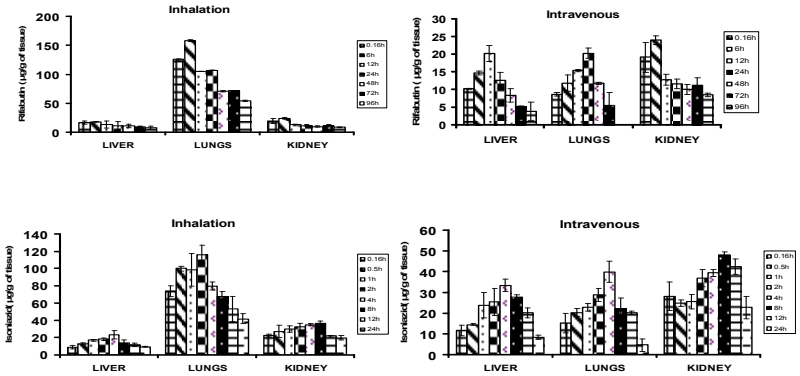

Figure 5: Tissue pharmacokinetics of isoniazid in lungs, liver and kidneys following intravenous injection (A) or inhalation of microparticles (C), compared to intravenous injection of rifabutin (B) or inhalation of microparticles (D)

This preferential selectivity of microparticles towards lung is attributed pulmonary route of administration and optimum aerodynamic diameter range of microparticle size i.e. 1-10µm which reaches to bronchi pathways and ultimately alveolar macrophages where mycobacterium resides. All the organs showed detectable levels of drug. Peak levels of isoniazid and rifabutin in lungs (target organ) were much higher than those in the liver and kidney of mice in case of inhalation as compared to intravenous administration. Inhalation of microparticles resulted in targeting both

drugs to the lungs, with the effect being more pronounced in the case of rifabutin than isoniazid. The relative bioavailability of both drugs incorporated in microparticles was significantly higher compared with free drugs. Pharmacokinetic parameters are shown in table-1 and 2.

Drug/ Route/ Organ	C_{max} ($\mu g.ml^{-1}$)	AUC_{OBS} ($\mu g.ml^{-1}.h^{-1}$)	$t_{½}$ (h)	V_z (ml)	Cl ($ml.h^{-1}$)
IV Lungs	8.16 ± 0.93	99.85 ± 14.24	6.45 ± 3.24	8.45 ± 3.80	0.96 ± 0.14
Liver	16.83 ± 1.61	246.86± 13.15	9.14 ± 1.12	4.35 ± 0.52	0.33 ± 0.00
Kidneys	8.54 ± 0.15	156.22± 7.57	23.25± 4.60	8.84 ± 0.65	0.38 ± 0.06
Inhalation Lungs	24.02 ± 1.71	566.31± 123.96	25.88± 12.16	190.11± 25.65	5.47 ± 1.30
Liver	12.12 ± 2.44	344.26± 57.08	25.86± 9.39	379.11± 14.68	9.16 ± 3.48
Kidneys	6.81 ± 0.30	237.26± 64.79	28.09± 12.12	379.43± 26.34	13.72± 4.88

Table 1: Pharmacokinetic parameters of Isoniazid in the lungs, liver and kidneys of mice after intravenous injection and inhalation of microparticles.

Drug/ Route/ Organ	C_{max} ($\mu g.ml^{-1}$)	AUC_{OBS} ($\mu g.ml^{-1}.h^{-1}$)	$t_{½}$ (h)	V_z (ml)	Cl ($ml.h^{-1}$)
IV Lungs	4.17 ± 0.31	187.63± 23.93	34.00± 3.31	16.78 ± 1.31	0.68 ± 0.45
Liver	9.76 ± 0.57	467.61± 33.97	49.72± 11.09	11.24 ± 2.25	0.15 ± 0.01
Kidneys	6.51 ± 0.86	392.40 ± 27.67	54.11± 13.55	13.34 ± 2.55	0.18 ± 0.01
Inhalation Lungs	33.42 ± 3.80	1697.39± 154.67	78.08± 9.42	131.33± 20.84	1.16 ± 0.22
Liver	9.52 ± 0.21	550.80 ± 17.24	117.6± 44.50	450.63± 66.95	3.05 ± 0.78
Kidneys	1.59 ± 0.01	255.73 ± 21.52	160.0± 69.51	1056.4± 214.10	2.93 ± 1.30

Table 2: Pharmacokinetic parameters of Rifabutin in the lungs, liver and kidneys of mice after intravenous injection and inhalation of microparticles

High and prolonged drug concentrations and increased AUC values (~9-fold and ~6 fold increase of rifabutin and isoniazid in case of lungs) with respect to free drugs were observed. Significant decrease in drug concentration was found in the liver and kidneys. These results are confirms that biodegradable microparticles are ideal for targeting and providing sustained release of anti-TB drugs to lungs. Targeted delivery of RFB to the lungs led to substantial reduction in first-pass metabolism of RFB. The findings suggest that polymeric microparticles prepared by spray drying process offer promises for treating pulmonary TB with reduced doses, lower dosing frequency and alleviated toxicity.

Conclusion

This study shows that drugs incorporated microparticles is able to deliver high concentration of drug and maintain therapeutic levels in lungs. Based on favorable biodistribution kinetics, these microparticles hold great potential in reducing dosing frequency and toxicity of anti-TB drugs.

References

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