PLA microparticles for pulmonary delivery of AntiTB drugs: biodistribution study

R. K. Verma, J. Kaur, A. B. Yadav, K. Kumar, A. Misra*

Pharmaceutics Division, Central Drug Research Institute, Lucknow- 226001 INDIA

Email-rahul_niper@yahoo.com

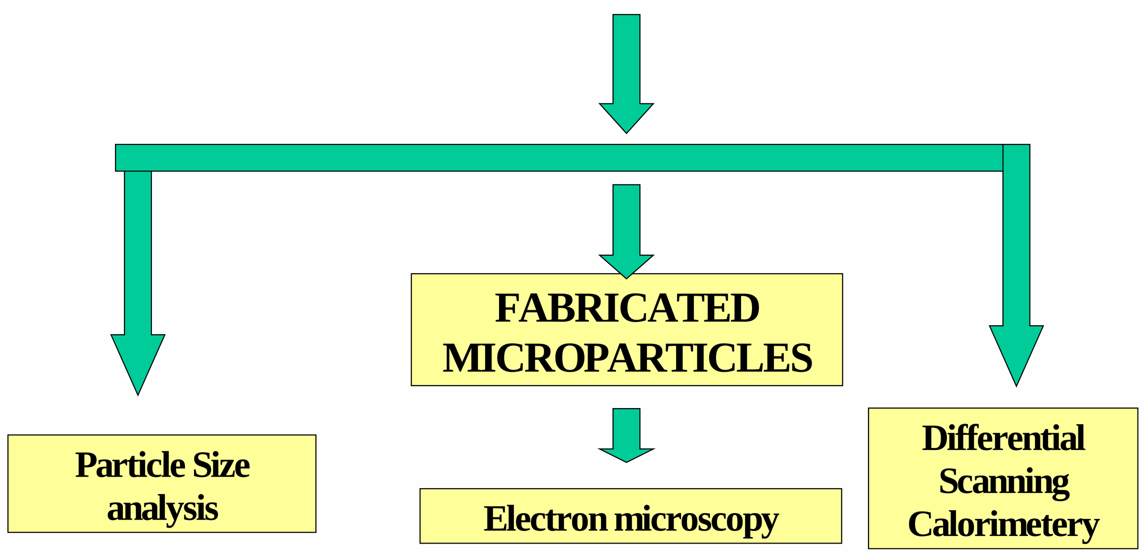
INTRODUCTION

Tuberculosis (TB) is a infectious disease caused by *Mycobacterium tuberculosis* (MTB). Among various forms of tuberculosis, pulmonary tuberculosis is the most common with the involvement of lung macrophages containing a large number of bacilli. Effective chemotherapy of pulmonary tuberculosis has been proposed through pulmonary delivery of biodegradable microparticles incorporating antituberculosis drugs. We have demonstrated administration of dry powder inhalations (DPI) of microparticles containing anti-TB drugs to various laboratory animals, which can target drugs to macrophages while decreasing bioavailability to blood and blood-perfused organs. The biodistribution of antituberculosis drugs has always remained a challenge and is responsible for the requirement of daily administration of these drugs during TB chemotherapy. In the present study, a DPI comprising anti-tuberculosis drugs incorporated in biodegradable microparticles was delivered by nose-only inhalation to mice. Poly L-lactic acid (PLA) microparticles incorporating a high payload of rifabutin and isoniazid were fabricated by spray drying to develop a sustained-release, macrophage-targeting delivery system containing the two drugs. Microparticles of desired high encapsulation efficiency and sustained release characteristics were produced having a diameter range of 1-10µm. Time-dependent biodistribution of rifabutin and isoniazid after single inhalation dose of drug loaded microparticles was established using a validated HPLC bioanalytical method. We studied the pharmacokinetics of the two drugs in target cells (alveolar macrophages) and lung tissue, as well as in the liver and kidneys, where their toxicity is most commonly manifested. Equivalent doses of free rifabutin and isoniazid were administered intravenously for comparison.

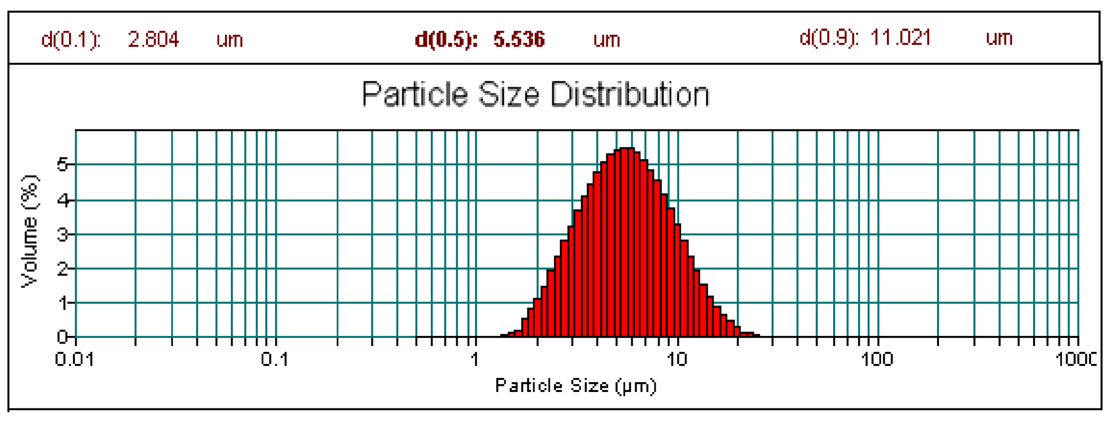
OBJECTIVES

- To prepare and characterize poly (D,L-lactic acid) microparticles containing rifabutin and isoniazid
- To evaluate the time kinetics of biodistribution of the two drugs i.e. Rifabutin and Isoniazid in the lungs, liver and kidneys of mice

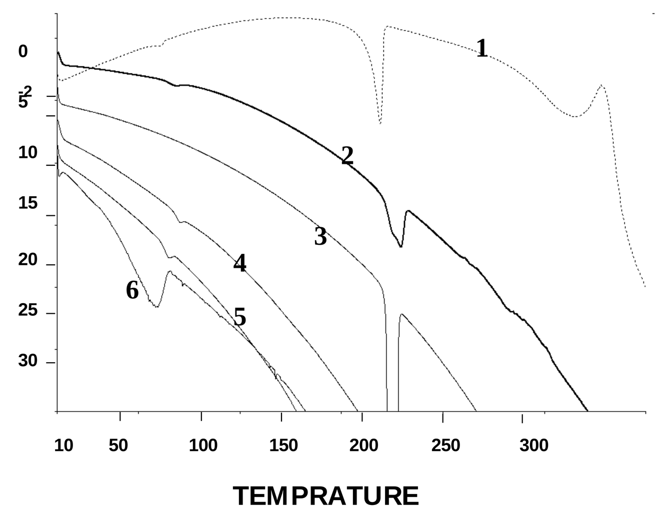
CHARACTERIZATION



PARTICLE SIZE DISTRIBUTION

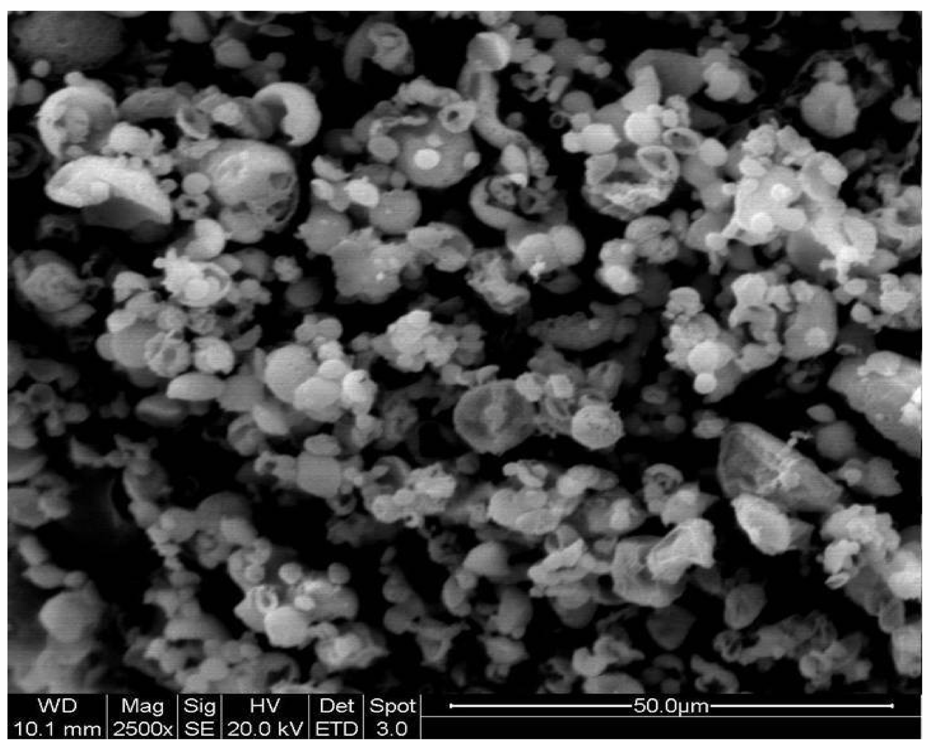


DIFFERENTIAL SCANNING CALORIMETRY



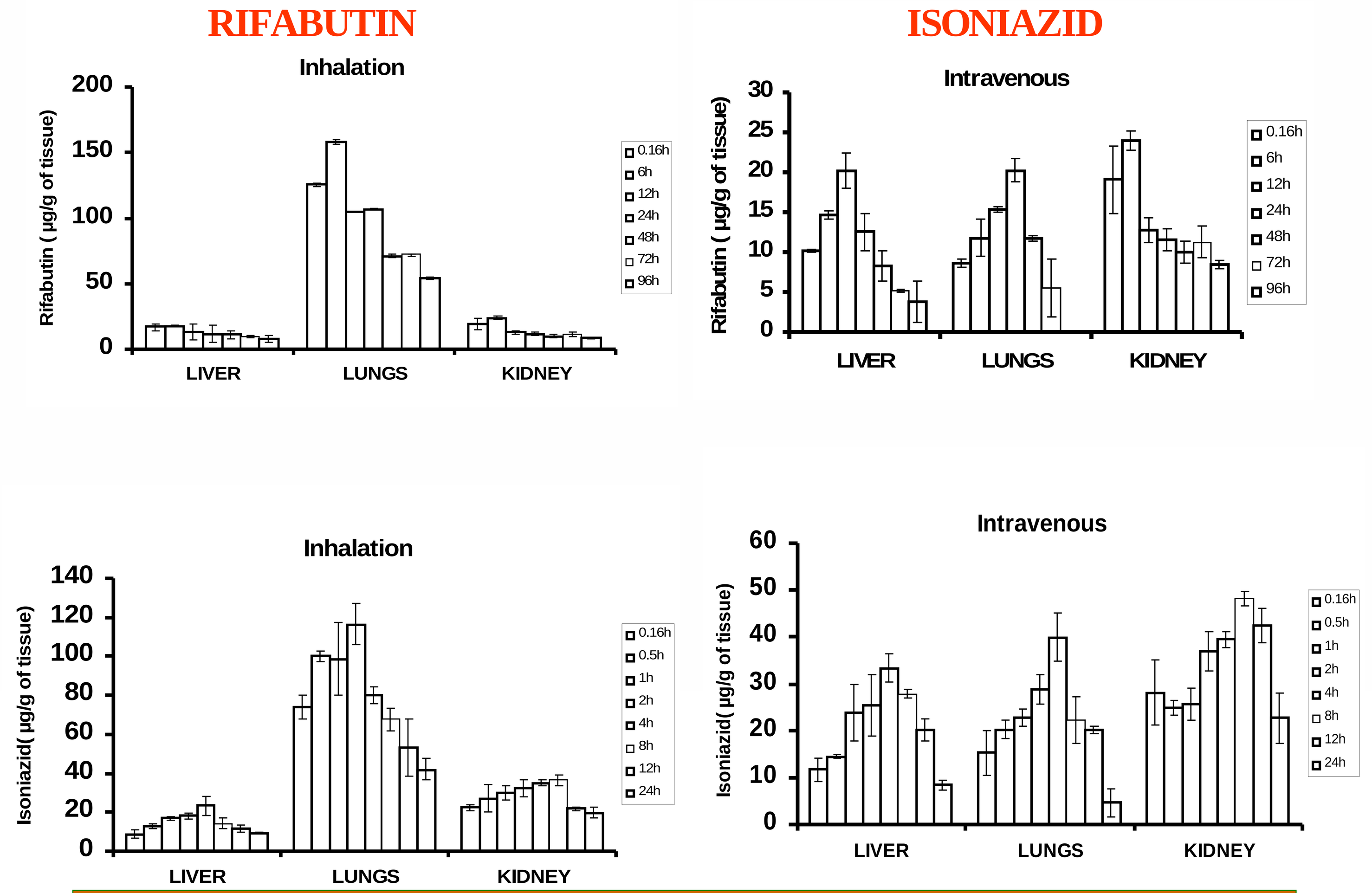
DSC thermograms showing melting temperature of PLA matrix(curve-2) containing Isoniazid(curve-1), Rifabutin(curve-6) and conbination of both (curve-5). Isoniazid(curve-3) and rifabutin (curve-4)

ELECTRON MICROSCOPY OF FABRICATED DRUG LOADED POLY LACTIC ACID MICROPARTICLES



INHALATION OF PLA MICROPARTICLES TO ANIMAL USING IN-HOUSE APPARATUS

BIODISTRIBUTION OF RIFABUTIN & ISONIAZID IN VARIOUS ORGANS OF MICE AT VARIOUS TIME INTERVALS (n=4)



PHARMACOKINETIC PARAMETERS OF RIFABUTIN & ISONIAZID IN THE LUNGS, LIVER AND KIDNEYS OF MICE AFTER INTRAVENOUS INJECTION AND INHALATION OF MICROPARTICLES

RIFABUTIN

Drug/ Route/ Organ	C_{max} (μ g.ml^{-1})	AUC_{OBS} (μ 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± 4.68	9.16 ± 3.48
Kidneys	6.81 ± 0.30	237.26± 64.79	28.09± 12.12	379.43± 6.34	ND

ISONIAZID

Drug/ Route/ Organ	C_{max} (μ g.ml^{-1})	AUC_{OBS} (μ 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 ±0.00

DISCUSSION

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

In the present study, inhalation of microparticles resulted in effective accumulation of the incorporated drugs in the lungs. At the same time, intravenous administration of equivalent amounts did not result in selective accumulation in targeted organ i.e. lungs.

All the organs examined showed detectable levels of drug. 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 of both the drugs to the lungs. The relative bioavailability of both drugs incorporated in microparticles was significantly higher compared with free drugs.

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 confirm that inhalable microparticles are suitable 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.

CONCLUSIONS

This study shows that drugs incorporated in microparticles generate high concentration and maintain therapeutic levels in lungs. Based on favorable biodistribution kinetics, these microparticles have the potential to reduce dosing frequency and toxicity of anti-TB drugs

ACKNOWLEDGEMENTS

NMITLI-CSIR for providing financial support for the project. ICMR for providing Senior research fellowship(SRF) to JK and CSIR for providing SRF to ABY