Microparticles specific for lung delivery: in-vivo drug distribution in monkeys

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INTRODUCTION

Inspite of extensive research in tuberculosis (TB), it is still one of the major infectious diseases and cause of high mortality world wide [*Brewer et al. 2005]*. To enhance efficacy with lower dosing, newer drug delivery systems has been developed which provide targeted drug delivery and controlled release at the primary site of infection, i.e., the lung macrophage. Our lab has previously reported *poly(*D, L-lactic acid) (PLA) microparticles containing rifabutin (RFB) and isoniazid (INH) for pulmonary delivery and explored various facets of treatment with microparticle DPI. We have utilized this dry powder aerosol drug delivery system to deposit drugs directly at the site of infection in lungs and have been investigating such DPI as a novel approach to increase therapeutic efficacy, patient compliance and lower toxicity. Data from murine models of DPI delivery suggests that aerosolized delivery of anti-TB drugs is more effective than conventional drug therapies, in maintaining therapeutic concentration at the site of infection [*Muttil et al. 2007*]. Most of the previous studies were primarily concerned with acute administration of drug-containing delivery systems and evaluation of their pharmacokinetic profile in rodents. The aim of present study was to evaluate pharmacokinetics, biodistribution and toxicity parameters of RFB and INH incorporated in DPI microparticles after ninety days of repeated dosing in rhesus macaques in order to establish steady-stae pharmacokinetics and preclinical safety of the formulation prior to humans clinical trials.

MATERIAL AND METHODS

Chemicals-Microparticles containing 25% RFB 25% INH and 50% *poly* (D, L-lactic acid) were donated by Lupin Laboratories Research Park, Pune, India. Dichloromethane, n-pentane, acetonitrile, methanol and solvents used in the experiments were of HPLC grade. ***Animals-***The animal study procedures were approved by the Institutional Animal Ethics Committee of the Central Drug Research Institute, Lucknow, India. Rhesus macaques (*Macaca mulatta*) of either sex with average weight 7.5 kg were bred, housed and fed ethically in the animal house of the Institute.

Animal dosing
Single dose inhalation- Four monkeys received a single dose 100 mg of microparticles by inhalation. About 500mg of drug loaded microparticles were charged and aerosolized by 30 actuation/ 30secs using an in-house apparatus reported earlier to obtain an inhaled dose of ~100 mg microparticles/animal. Before dosing, the monkeys were trained for 30 days to accept restraint and application of an infant inhalation mask attached to our in-house apparatus. ***Repeated dose intravenous-*** Four monkeys received repeated daily dosing of 100 mg of microparticles, 5 days a week for a total of 90 days. The dose interval was 24 h. ***Single dose intravenous-*** Monkeys were administered single dose intravenous injection of a combination of 25mg each of RFB and INH in DMSO and sterile Phosphate buffer saline (PBS) solution. Blood sampling through the cubital a greater sphenoid veins was done at indicated time intervals

Sample collection
Blood- For single-dose inhalation pharmacokinetics study, monkeys were sampled at different time intervals *viz,* before dosing, 10 min after dosing, and then 1, 2, 4, 8, 12, and 24h after inhalation. Similarly, for multiple- dose inhalation pharmacokinetics, monkeys were sampled on day-60 at the same time intervals. ***Tissues-***After 90 days of repeated dosing, animals were euthanized by overdose of anesthesia (thiopentone). Tissues of interest i.e. lungs, liver and kidneys were excised blotted dry, weighed and kept in -20ºC for further analysis. ***Bronchoalveolar lavage-***After sacrifice, thoracic cavities of animals was opened; lungs intact with trachea were excised. The trachea was cannulated and lungs were repeatedly lavaged with chilled PBS (containing 0.5 mM EDTA). Bronchio-alveolar lavaged fluid (BALF) was pooled, centrifuged and macrophages obtained were counted and kept at -20°C for further analysis.

Sample, processing and bioanalyis
INH was extracted from serum and tissues by the method described by *Hutchings et al*. using chloroform: butanol (70:30v/v) and analysed by HPLC on a C-18 columnin using a mobile phase consisting of triethyl amine acetate (TEA buffer) and acetonitrile (97:3 v/v). at 262nm. RFB was extracted using dichloromethane: *n-P*entane (1:1 v/v) and eluted from the same column using a mobile phase of phosphate buffer-Acetonitrile (45:55) with detection at 275nm. Descriptive statistical analysis (i.e., means ± standard deviation) was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA). Time-courses of serum drug concentration following inhalation and intravenous administration were analyzed for pharmacokinetic parameters using WinNonLin pharmacokinetic computer modeling program (Pharsight Corp., Carry, NC).

RESULTS AND DISCUSSION

Prepared microparticles formulation has physicochemical properties that render it suitable for administration as a dry powder inhalation (DPI). Primary among these are the particle size distribution and median diameter

Fig 1 : Dosing of monkeys using In-house nose-only inhalation apparatus

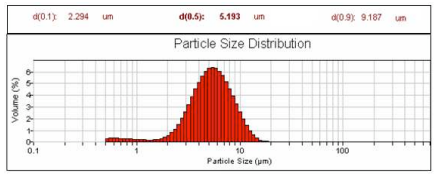

Figure 2: Particle size distribution of microparticles obtained by Spray drying technique

in suspension, median mass aerodynamic diameter (MMAD) and the geometric standard deviations from (GSD) from this median, and relative fine particle fraction (FPF). These are illustrated in Figures 2, 3 and 4. Serum was separated from blood samples of each time point and analyzed by HPLC.Plots representing the mean drug serum concentration–time data after intravenous administration of rifabutin and isoniazid to monkeys are shown in figure.6

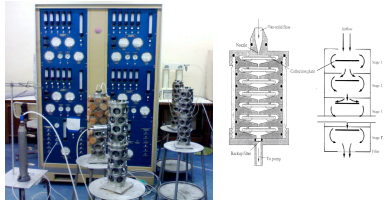

Fig 3: Determination of aerosol characteristics .i.e Mass Median Aerodynamic Diameter (MMAD) using InTox inhalation assembly and Seven stage cascade impactor

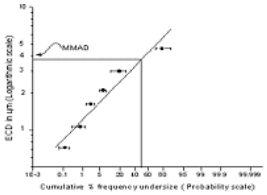

Figure 4: Microparticles deposited on each collection disc of cascade impactor plotted against effective cut-off diameter on log probability

Compartment modeling could not be fitted over the data obtained on analysis of serum after inhalation; hence, Non-compartmental analysis was performed. In contrast to inhalation, i.v. administration resulted in hasty clearance of drugs from the blood, significantly shorter half-lives and mean residence times.

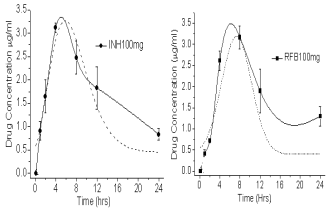

Figure 5: Serum concentration of drug in monkeys receiving *intravenous administration* 25mg of each drug (equivalent to 100mg of microparticles)

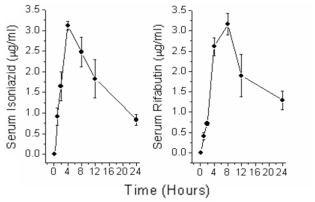

Fig 6: Serum concentration of INH and RFB in monkeys receiving *Single dose* of 100 mg of inhalable microparticles

Dose-dependant enhancement of all parameters was observed, as expected from the order of magnitude differences in doses. After 90-day study, various tissues of interest (lung, liver and kidney) were harvested from twenty monkeys, minced into pieces, homogenized and analyzed for drug content. It could be seen from figure-8 that concentration of both the drugs descends in order of lungs, liver and kidney. As evident from figure, level of rifabutin and isoniazid in lungs were much higher than tissue than other tissues.

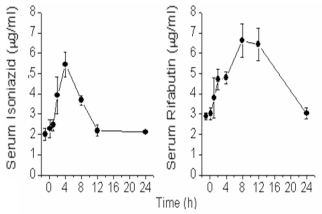

Figure 7: Serum concentration of INH and RFB in monkeys receiving *repeated dose* of 100 mg of microparticles on day 60

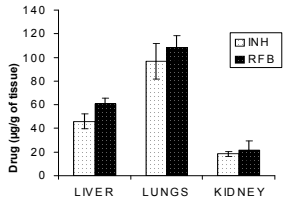

Fig 8: *In-Vivo* distribution of INH and RFB in monkeys after receiving *daily administration* of 100 mg microparticles

There was no significant difference in any of the parameters tested between the exposed mice as compared to the controls after 90 days exposure to the microparticles containing drug formulation.

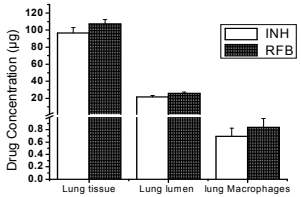

Fig 9: Drug distribution in various parts of Lungs of rhesus monkey after 90 day repeat dose of 100 mg of microparticles

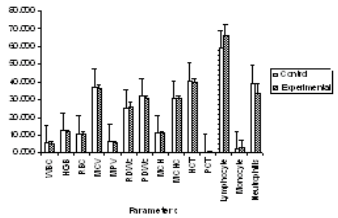

Fig 10: Hematological parameters of control and exposed animals during the sub-chronic study in monkeys

CONCLUSIONS

The results of this report suggests microparticles lead to the deposition of drugs into the lungs by means of inhalation and maintain high therapeutic concentration which undoubtedly improve pulmonary tuberculosis chemotherapy. The data obtained from rhesus monkeys with good therapeutic concentration in target organ and negligible toxicity (data not shown) strongly recommends for the further human clinical trails

REFERENCES

• Brewer et al. (2005) *Long time due: reducing tuberculosis mortality in the 21st century.* Arch Med Res 36(6) 617-621
• Muttil et al. (2007) Inhalable microparticles containing large payload of anti-tuberculosis drugs. Eur J Pharm Sci 32(2) 140-150