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Macrophage Targeted Nanomedicines for Mycobacterial Infections

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Abstract

Eradication of Mycobacterium Tuberculosis (MTB) infection commonly requires combination daily administration of rifampin, isoniazid, pyrazinamide, ethambutol amongst others to achieve microbial eradication. To facilitate and optimize therapeutic outcomes, we developed a mononuclear phagocyte (MP; monocyte, macrophage and dendritic cell) drug carriage system. MP serves as the natural cell reservoir of MTB facilitating nanoformulated drug particle carriage. To this end, the physical and chemical properties of nanoformulated rifampin (RIF) and isoniazid (INH) pentenyl-derivative (INHP) were evaluated and resultant particle MP uptake, retention, viability and antimicrobial efficacy were determined. Up to 6 µg of INHP and RIF/10^6 human monocyte-derived macrophages (MDM) were taken up as compared to 0.1 µg/10^6 cells for native drug. Sustained release of nanoformulated INHP and RIF were present in MDM at 15 days whereas native drugs were released within 24 hours. Antimicrobial activities determined in dose response tests against *Mycobacterium smegmatis* (*M. smegmatis*) showed a 6-fold increase over equivalent concentration of native drug when both INHP and RIF nanoformulations were administered. Importantly, the drug nanoformulations were found cloistered into the recycling and late endosomes, the identical subcellular compartments, known to contain the replicating microbial pathogens. These results highlight new opportunities for antimicrobial nanomedicines to affect eradication of MTB in an infected human host.

Introduction

Despite the broad availability of antituberculin therapies, MTB remains a major cause of worldwide severe disease morbidities and death. Currently available drugs used to treat MTB require long therapies with no treatment interruptions. This ensures that the mycobacterium are targeted and antimicrobial activities are sustained [1]. Secondary drug toxicities and effective penetrance of drugs into microbial cell reservoirs compound therapeutic efficacy, as does the emergence of drug-resistant organisms. We reasoned that this would be improved by the use of therapeutic targeting to MP [2]. To this end a monocyte-macrophage drug targeting system for rifampin (RIF) and isoniazid (INH) was developed facilitating drug cell uptake while maintaining intracellular drug levels.

Materials and Methods

Nanoparticle synthesis: The Poly-lactide-coglycolic acid (PLGA) nanoparticles containing either isoniazid derivative (INHP) [3] or rifampin (RIF) were separately prepared by double emulsification using sonication. The particle size was measured using Zetasizer and drug concentrations were determined by reversed phase high performance liquid chromatography (RP-HPLC).

Cell culture and measurement of antimicrobial activities: Human monocytes were collected by leukapheresis [4]. After seven days of in vitro culture in macrophage colony stimulating factor (MCSF) monocyte-derived macrophages (MDM) were treated with nanoformulations and native drugs. Uptake and retention were determined by RP-HPLC. At days 1, 5 and 10 following drug treatment, the MDM were infected with *M. smegmatis* (MOI = 1). The cell suspension was treated with 0.25% of SDS solution then loaded on agar plates. After 3 days of incubation, the number of colony forming units were counted [5].

Endocytic trafficking measurements: For confocal microscopy, MDM were adminstered 300 µM of either fluorescein labeled RIF or INHP nanoparticles. The cells were then incubated with RAB5 (early endosome), RAB7 (late endosomes), RAB11 and RAB14 (recycling endosomes). The cells were then washed and incubated with the secondary antibody conjugated to Cruz Fluor 488.
For immunoisolation of endocytic compartments [6] drug content of each compartment was determined by RP-HPLC.

Results

PLGA nanoformulations showed enhanced uptake of up to six µg of INHP and RIF/10^6 cells when compared to 0.1 µg/10^6 cells for native drugs.

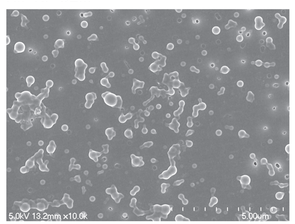
Sustained release of encapsulated INHP and RIF were present in MDM over 15 days whereas native drug was released within 24 hours.

Antimicrobial activities as determined in dose response tests against *M. smegmatis* showed ~6-fold increase over equivalent concentration of native drug when both INHP and RIF nanoformulations were administered.

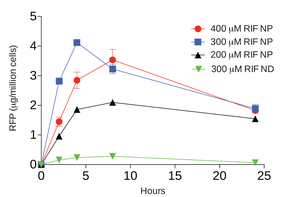
Endocytic trafficking of the antimycobacterium nanoparticles compared side-by-side with *M. smegmatis* showed identical subcellular compartment localization in recycling and late endosomes.

SEM Images, Nanoparticle Uptake and Retention

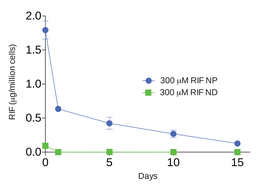
RIF NP SEM



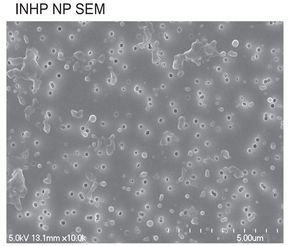
RIF dose response uptake



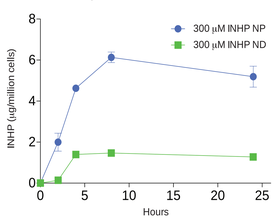
RIF retention



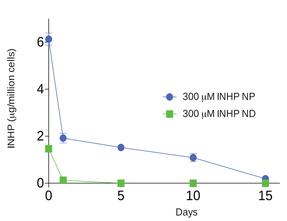
INHP NP SEM



INHP uptake

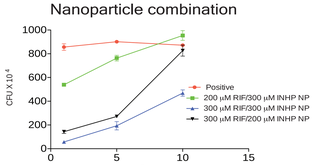


INHP retention

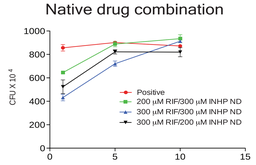


Antimicrobial Activity: Native drugs vs. Nanoformulations

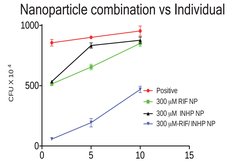
Nanoparticle combination



Native drug combination



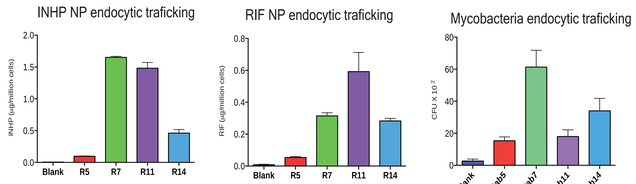
Nanoparticle combination vs Individual drugs



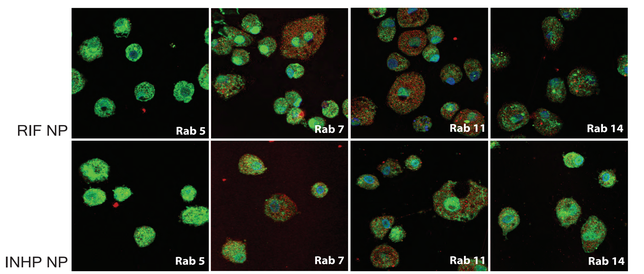
Acknowledgements

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Subcellular Distribution of the Nanoparticles and *M. Smegmatis*



Subcellular Localization of the Nanoparticles by Confocal Microscopy



Co-localization of PLGA-RIF and PLGA-INHP with subcellular compartments. Nanoparticles are shown in red, cell compartments in green, and nuclei in blue.

Conclusions

Nanoparticles encasing RIF and INHP antitubercular therapies were successfully synthesized.

PLGA nanoparticles encapsulating RIF and chemically modified INH derivative improves drug uptake, retention and antimicrobial efficacy of the nanoformulations in MDM when compared to the native drugs.

Confocal data supported by endocytic trafficking of *M. smegmatis* and the PLGA nanoformulations revealed that they both interact at the subcellular level thereby enhancing their antimicrobial efficacy.

Macrophage-nanocarrier drug delivery approach is a promising system that would improve outcomes in tuberculosis therapy

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