

Improving the in vitro pharmacokinetic profile of an existing malaria and tuberculosis drug using nanotechnology

P. N. Fonteh¹, R. Hayeshi², D. Meyer¹, H. Swai²

¹University of Pretoria, Biochemistry, Pretoria/ZA

²Council for Scientific and Industrial Research, Materials Science and Manufacturing, Polymers & Composites, Pretoria/ZA



Pascaline Fonteh

Dr. Pascaline Fonteh obtained her Ph.D. in Biochemistry from the University of Pretoria (UP) in 2012, focusing on the anti-HIV and immunomodulatory activity of gold-based compounds. She did a postdoctoral fellowship through UP and the South African Council for Scientific and Industrial Research and currently holds a research position at the University of the Witwatersrand where she is an investigator on translational research projects. Her current focus is on using polychromatic flow cytometry in studying immune parameters to predict prognosis and in screening novel small molecules as potential inhibitors or immunotherapy for pancreatic disease patients co-infected with HIV.

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

Introduction

Drug toxicity and the resultant poor treatment compliance for poverty related disease (PRD) drugs such as those used in the treatment of HIV, malaria and TB are some of the limitations hindering treatment success. Nanotechnology-based drug delivery is one way of circumventing this. Here, an immunostimulant nanocarrier, curdlan poly(D,L-lactide-coglycolide) (C-PLGA) was synthesised and tested for improved delivery of rifampicin on Caco-2 cells. Conjugation of the malaria drug, primaquine (PQ) to a macromolecular polymer and subsequently testing for toxicity was also undertaken. A more effective immunostimulant PLGA nanocarrier was successfully synthesized and characterized resulting in improved uptake of rifampicin. The hemolytic effect common with PQ was alleviated upon conjugation with the macromolecular polymer. In conclusion, an immunostimulant C-PLGA nanocarrier which could potentially lead to the development of multifunctional nanomedicines with dual properties capable of immune stimulation and drug delivery was synthesized. For PQ the intrinsic hemolytic effect was removed through conjugation with a macromolecular polymer. Such a drug entity should eventually alleviate the problem of hemolysis associated with PQ.

Background and Justifications

Sub-Saharan Africa bears the greatest burden of poverty related diseases (PRDs) such as tuberculosis (TB), malaria and HIV (1). Existing drugs for these PRDs are limited in efficacy due to poor PK and the development of drug resistance by the pathogens involved. Toxicity to the host is also common and results in patient non-compliance such that suboptimal treatment responses (failure and relapse) occur, further enhancing drug resistance. In the case of TB for instance, treatment is lengthy because most of the drugs are characterized by a short half-life and limited solubility in biological media. Ultimately, for TB and drugs for other PRDs, which have similar limitations, high doses and dose frequencies are required due to the associated poor bioavailability, further complicating the side effects (2).

While statistics indicate an urgent need for the development of novel and/or improved drugs, the investment allocated for the research and development (R&D) of these drugs is woefully inadequate. Pharmaceutical companies have lagged in the discovery of drugs for the

continued on next page



ISID Report of Pascaline Fonteh

Improving the in vitro pharmacokinetic profile of an existing malaria and tuberculosis drug using nanotechnology

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Research Grant Report *continued*

diseases of the developing world due to the cost of R&D, the risk involved and the timeconsuming nature of this field. As a result, with respect to the discovery and development of drugs against PRDs, where minimal (if any) returns can be expected; new approaches such as those involving nanotechnology and nanomedicine must be explored.

Nanotechnology-based drug delivery systems (nanomedicine) offer a possible solution by presenting the ability to alter the PK of conventional drugs or promising new molecules to enhance their bioavailability, increase half-life and reduce toxicity. The technology involves nano-encapsulating drugs using nano-sized carrier systems such that the active agent present in the drug is delivered to the target tissue or microenvironment in a controlled and sustained manner. Nanomedicine has already shown success for anti-cancer agents with several anti-cancer drugs approved by the Food and Drug Administration (3). Unfortunately, the technology has not been widely explored for PRDs by pharmaceutical companies whose focus is commercially driven. This study was conducted at the University of Pretoria in collaboration with the African Network for Drugs and Diagnostics Innovation (ANDI) Pan-African Centre of Excellence (CoE) in Nanomedicine, housed at the CSIR. The CoE had previously used TB as a model for enhancing the efficacy of existing PRD drugs (4, 5).

The CoE has already nano-encapsulated four TB drugs with an encapsulation efficiency of 50–65%. Successful intracellular delivery was reported for these agents *in vitro* and slow release and reduced dose frequency potential shown *in vivo* (6). Based on the progress made with TB, the CoE aimed at optimising the formulations better and at expanding into encapsulating drugs for HIV, malaria and other neglected diseases. The overall goal was to develop a PK/PD platform which will be instrumental in supporting the development of nanomedicines for PRDs. PK defines the absorption, distribution, metabolism and excretion (ADME) profiles of the drugs and PD provides information on the relationship of the drug concentration at the site of action.

Efforts in understanding the PK of nanocarriers are on the increase, however, due to the great diversity in nanocarrier properties and experimental design, data cannot be compared between studies. In addition, the majority of nanomedicines being developed are for intravenous delivery, whereas the CoE is developing nanomedicines for the less costly and user friendly oral delivery route. This makes the development of an in-house, PK/PD model for these nanocarriers important since it will potentially support nanomedicine drug development for PRDs.

In an effort to do develop this platform, we synthesized a novel polymer consisting of PLGA) conjugated to curdlan and tested this carrier for rifampicin across Caco-2 cells. The malaria drug, primaquine was conjugated to a macromolecular polymer (name withheld) and tested for reduced toxicity.

Primary Objectives

- 1) Synthesis and characterize of novel carriers



ISID Report of Pascaline Fonteh

Improving the in vitro pharmacokinetic profile of an existing malaria and tuberculosis drug using nanotechnology

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Research Grant Report *continued*

- 2) Test for improved dissolution and release kinetics of the drugs.
- 3) Determine cellular toxicity.
- 4) Perform permeability studies for evaluation of nanocarrier absorption and transport.
- 5) Determine the metabolism of the nanomedicine formulations by cytochrome P450 enzymes.
- 6) Correlate *in vitro* drug release to *in vivo* drug release

Summary of Findings

Novel polymer synthesis and drug delivery studies with rifampicin: A publication detailing the synthesis, characterisation and immunostimulant properties of a novel C-PLGA polymer was published last year (7). In the paper, carbodiimide chemistry was employed to conjugate curdlan to PLGA followed by spectral characterisation. Nanoparticles of the C-PLGA polymer were synthesized using an emulsion-solvent evaporation technique. Immunostimulatory activity was characterized in THP-1 derived macrophages. A tetrazolium dye assay and realtime impedance measurements were used to characterize polymer and nanoparticle toxicity and uptake in macrophages. Drug delivery capability was assessed across Caco-2 cells using rifampicin, an anti-tuberculosis agent as a model drug. Spectral characterization confirmed successful synthesis of Curdlan-poly(D,L-lactide-co-glycolide) (C-PLGA). C-PLGA nanoparticles enhanced phosphorylated ERK production in macrophages indicating cell stimulation. Nanoparticles provided slow release of rifampicin across Caco-2 cells (model for intestinal uptake and permeability studies). Impedance measurements suggested Ca²⁺ dependent uptake of nanoparticles by the macrophages. The findings confirm successful synthesis of PLGA nanoparticles with macrophage stimulating and sustained drug delivery capabilities. The nanoparticles can be used to stimulate macrophages and concurrently deliver drug in infectious disease therapy.

Primaquine macromolecular polymer toxicity and hemolysis studies: Another approach to improving the delivery of existing drugs has been the conjugation of the malaria drug, primaquine to a macromolecular polymer. PQ is the only FDA approved drug against the liver stage and systemic circulation of the gametocyte stage of the malaria parasite. Unfortunately, it is an 8-aminoquinoline which is a class of drugs known for their hemolytic toxicity in glucose-6-phosphate dehydrogenase (G6PD) deficient patients. G6PD deficiency is an inborn error of metabolism disease prevalent among the population in malaria endemic regions (8). This shortfall of the drug has limited its clinical use. In an attempt to reduce this limitation, several investigators have conjugated PQ to polymeric carriers. The chemical conjugation of the drug to a macromolecular polymer results in a pro-drug with new physico-



ISID Report of Pascaline Fonteh

Improving the in vitro pharmacokinetic profile of an existing malaria and tuberculosis drug using nanotechnology

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Research Grant Report *continued*

(bio)chemical attributes. This new chemical entity has some predictable physical properties like solubility which are influenced mainly by the much larger molecular weight polymer. The resultant new chemical compound (1), with multivalent functional groups was evaluated for cytotoxicity and effect on hemolysis in comparison with the free drug and the macromolecular polymer. A pH dependent hemolysis of red blood cells and cytotoxicity of the conjugate and free drugs was determined on primary cells and the CaCo-2 cell line using the method by (9). The free drug was found to be more toxic than the conjugate and to result in more significant hemolysis especially at the acidic pH of 5.6 than the drug conjugate. These findings suggest that conjugation of PQ to macromolecular polymer could alleviate the hemolytic effect common with this class of drugs and potentially result in better patient outcome. The findings from this work are currently being compiled for publication.

An abstract for both studies entitled: "Improving the in vitro pharmacokinetic profile of an existing malaria and TB drug using nanotechnology" was submitted and accepted for presentation at the 17th International Congress for Infectious Diseases that was held in Hyderabad, India from the 2-5th of March 2016. Because we want to publish the data, embargo reasons let us to withdraw the abstract. We are currently busy with the publication.

Study Limitations

Internal restructuring at the CSIR and funding limitations meant all aspects of the study could not be finalized e.g. permeability studies on Caco-2 cells. This also limited work on cytochrome metabolism studies, animal PK and tissue distribution. Encapsulation of the proposed HIV compound was also not explored as a result.

Research Highlights and Conclusions

To develop nanoparticles drugs with improved pharmacokinetic and pharmacodynamics properties, an optimized carrier for these drugs was developed. A novel C-PLGA carrier was produced. This nanoparticle provided slow release of rifampicin, a model TB drug, across Caco-2 cells. Impedance measurements suggested Ca²⁺ dependent uptake of nanoparticles by the macrophages. The findings confirm successful synthesis of PLGA nanoparticles with macrophage stimulating and sustained drug delivery capabilities.

PK studies for the malaria drugs, PQ was studying by looking at the effect of minimizing hemolysis after conjugation. The hemolytic effect common with PQ was alleviated after conjugation with a macromolecular polymer. This conjugation thus produces a drug that could potentially result in better patient outcome. This data is being compiled for publication.

References

1. Vitoria, Marco, et al. "The global fight against HIV/AIDS, tuberculosis, and malaria." *Am J Clin Path.* 131.6 (2009):



ISID Report of Pascaline Fonteh

Improving the in vitro pharmacokinetic profile of an existing malaria and tuberculosis drug using nanotechnology

This research was supported with a grant from the International Society for Infectious Diseases (ISID).

ISID Research Grant Report *continued*

844-848.

2. Hayeshi et al., in Chibale et al., (eds.) 2012, Drug Discovery in Africa, pg 407.

3. Alexis, Frank, et al. "New frontiers in nanotechnology for cancer treatment." *Urologic Oncology: Seminars and Original Investigations*. Vol. 26. No. 1. Elsevier, 2008.

4. Semete, Boitumelo, et al. "In vivo evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems." *Nanomedicine*: 6.5 (2010): 662-671.

5. Semete, B., et al. "In vivo uptake and acute immune response to orally administered chitosan and PEG coated PLGA nanoparticles." *Toxicol. Appl. Pharmacol* 249.2 (2010): 158-165.

6. Semete, Boitumelo, et al. "Potential of improving the treatment of tuberculosis through nanomedicine." *Mol. Cryst. Liq. Cryst* 556.1 (2012): 317-330.

7. Tukulula M., Hayeshi R., Fonteh P., et al. "Curdlan-conjugated PLGA nanoparticles possess macrophage stimulant activity and drug delivery capabilities." *J Pharm Res* 32 (2015): 2713-2726.

8. Luzzatto, Lucio, and Elisa Seneca. "G6PD deficiency: a classic example of pharmacogenetics with on going clinical implications." *Br J Haematol* 164.4 (2014): 469-480.

9. Evans, Brian C., et al. "Ex vivo red blood cell hemolysis assay for the evaluation of pHresponsive endosomolytic agents for cytosolic delivery of biomacromolecular drugs." *JoVE* 73 (2013): e50166-e50166.