UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Assessment of mycolic acids as ligand for nanoencapsulated anti-tuberculosis drug targeting

Yolandy Lemmer

Submitted in partial fulfillment of the degree

***Philosophiae Doctor* Biochemistry**

in the

Department of Biochemistry

Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

October 2010

Submission declaration:

I Yolandy Lemmer declare that the thesis which is herewith submitted to for the degree *Philosophiae Doctor* Biochemistry at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Plagiarism Declaration

UNIVERSITY OF PRETORIA

FACULTY OF NATURAL AND AGRICULTURAL SCIENCES

DEPARTMENT OF BIOCHEMISTRY

Full name: _____ Student number: _____

Title of the work: _____

Declaration

1. I understand what plagiarism entails and am aware of the University's policy in this regard.
2. I declare that this _____ (e.g. essay, report, project, assignment, dissertation, thesis etc) is my own, original work. Where someone else's work was used (whether from a printed source, the internet or any other source) due acknowledgement was given and reference was made according to departmental requirements.
3. I did not make use of another student's previous work and submit it as my own.
4. I did not allow and will not allow anyone to copy my work with the intention of presenting it as his or her own work.

Signature _____ Date _____

Acknowledgements

I would like to thank the following individuals and organizations for their support towards the completion of my PhD degree.

Most importantly, our **Creator and King**, for the guidance and support in the good and trying times and never leaving my side.

Prof. J.A. Verschoor my promoter for all my post graduate studies, for his door always being open whether it is science related or not. For all the interesting discussions, valuable input, positive criticism and for guiding me in the writing of my thesis.

Dr. Hulda Swai my promoter at the CSIR, for allowing me to be part of her research group and giving me the opportunity to study abroad, as well as all the valuable life lessons I have learned during our motivating discussions.

Dr. Boitumelo Semete-Makokotlela my co-promoter at the CSIR, for assisting me in my experimental planning and helping me to make it work, as well as the excellent help and contribution in the writing of my thesis - without your help it would not have been successful.

Dr. Arwyn Tomos Jones my supervisor at Cardiff University, without whom I would have never successfully done all the *in vitro* studies.

Prof. Dave G. Fernig my supervisor at University of Liverpool, who assisted me in the biosensor experimental work.

Dr. Bienyameen Baker and the University of Stellenbosch for the use of their facilities.

Very special thanks to **Mrs. Sandra van Wyngaardt**, for going out of your way to help me in the lab even in the late hours of the night especially the bacterial experiments and most of all being my second mother.

The BIA group, for all the valuable suggestions and help in my work and always making me feel welcome.

All the individuals in the **TB research group** at the CSIR, for their help with experiments, suggestions and tolerating my inquisitiveness.

A special thanks to **Mr. Lonji Kalambo** for his excellent ideas and help in the particle synthesis. **Mrs. Laetitia Booysen** and **Mrs. Ilse Wepener** for assisting me in my cell culture work as well as being my friends. I would also like to thank **Miss Bathabile Ramalapa** for her technical assistance with the SEM analyses and **Mr. Wayne Barnes** for his technical assistance in the FACS experiments. To my friend and colleague **Mr. Chrisitan Stutzer** for all the good suggestions and revisions.

I would also like to thank, **NRF SA-UK Bilateral** fund for financial support, **CSIR** for the studentship and infrastructure, **DST** for supporting infrastructure and the **Marie Curie fellowship**.

My family and friends, for their moral support and always trying to understand. Special thanks to my **mother** and **father** for the moral support and encouragements. My twin sister **Xelia**, for always being there and being my best friend. Thank you to **Marshant** for being such a good listener and brother. Lastly, I would want to especially thank **Corné**, words cannot express the gratitude I have and how deeply I appreciate all that you have done, truly my better half.

Table of contents

List of abbreviations

AD/ADD	4-androstene-3,17-dione/1,4androstadiene-3,17-dione pathway
AIDS	acquired immune deficiency syndrome
5BMF	5-Bromomethylfluorescein
Ca^{2+}	calcium
Caco-2	human colorectal carcinoma cell line
CIP	coronin interactin protein
CSIR	Council for Scientific and Industrial Research
DCM	dichloromethane
$dddH_2O$	double distilled deionized water
DIM	Phthiocerol dimycocerosates
DOT	Directly Observed Treatment
EE	encapsulation efficiency
EEA1	Early endosomal antigen 1
EIPA	5-N-ethyl-N-isopropylamiloride
EO	ethylene oxide
EPR	enhanced permeation and retention
ER	endoplasmic reticulum
FACS	fluorescence activated cell sorting
FAS	fatty acid synthetases
FCS	foetal calf serum
Fe_2O_3	ferrous oxide

GI	bacterial growth index
HASMC	human arterial smooth muscle cells
HIV	human immunodeficiency virus
HPLC	High Performance Liquid Chromatography
IFN-γ	interferon-gamma
Igr	intracellular growth
IL-12	interleukin 12
INH	isoniazid
LAM	lipoarabinomannan
LD	lipid droplet
LpdC	lipoamide dehydrogenase C
LXR	liver X receptor
MA	mycolic acids
ManLAM	mannose-capped lipoarabinomannan
MARCO	macrophage receptor with collagenous structure
M. avium	*Mycobacterium avium*
MDR-TB	multidrug resistant tuberculosis
M. leprae	*Mycobacterium leprae*
MOI	multiplicities of infection
MS	mass spectrometry
M.tb	*Mycobacterium tuberculosis*
mV	milli-volts
MW	molecular weight

nm	nanometer
NMR	nuclear magnetic resonance
NP	nanoparticles
PAC	poly-alkyl-cyano-acrylate
PAMP	pathogen associated molecular patterns
PBS	phosphate buffer saline
PCL	poly-ε-caprolactone
PDI	polydispersity index
PEG	polyethylene glycol
PFA	paraformaldehyde
PIM	phosphatidylinositol-mannoside
PI(3)P	phosphatidylinositol-3-phosphate
PI(3)K/Vps34	phosphoinositide 3- kinase
PLA	polylactic acid
PLGA	poly, DL, lactic-co-glycolic acid
PMA	phorbol 12-myristate 13-acetate
PO	propylene oxide
PVA	polyvinyl alcohol
RIF	rifampicin
RSA	Republic of South Africa
rpm	revolutions per minute
RT	room temperature
SapM	*M. tuberculosis* effector molecule

SEM	scanning electron microscopy
S1P	sphingosine-1-phosphate
SiO_2	amorphous silica oxide
SLN	solid lipid nanoparticles
TACO	tryptophane aspartate containing coat protein
TB	tuberculosis
TDM	trehalose dimycolate
THP-1	myelomonocytic cell line
TLC	thin layer chromatography
TLR	toll-like receptors
TMM	trehalose monomycolate
TNF-α	tumour necrosis factor alpha
TraSH	transposon site hybridization
U937	histiocytic lymphoma
USFDA	United States food and drug administration
WHO	World Health Organisation
w/o	water in oil
w/o/w	water in oil in water
WST	tetrazolium salt
w/v	weight/volume
XDR-TB	extensively drug resistant tuberculosis
ZnO	zinc oxide

List of figures

Summary

South Africa currently has the highest incidence of TB per 100 000 people in the world. In 2007 alone 112 000 people died of TB in South Africa, of which 94 000 were co-infected with HIV. Although TB treatments exist, poor patient compliance and drug resistance are challenges to TB management programs worldwide. Here, this challenge was addressed by the development of a polymeric anti-TB nanodrug delivery system for anti-TB drugs that could enable entry, targeting and sustained release for longer periods, hence reducing the dose frequency and simultaneously improve patient compliance. The aim was to prepare functionalised polymeric nano drug delivery vehicles to target TB infected macrophage cells. Successful nano encapsulation of anti-TB drugs was achieved and uptake of the antibiotics in the cells, demonstrated. A possible targeting agent, mycolic acids (MA) from *M. tuberculosis* was explored. The MA incorporated into nanoparticles could possibly serve as a ligand for cholesterol-rich areas, due to the cholesteroid nature of MA and the fact that MA is attracted to cholesterol. In another targeting scenario, MA incorporated into nanoparticles may interact with the anti-mycolic acid antibodies that are anticipated to be present in higher concentrations at the infected areas. The cholesteroid nature of MA was confirmed and how it related to the fine structure of the MA. The prepared MA containing nanoparticles were shown *in vitro* to be taken up in macrophage cell lines, without the MA hindering the uptake of the particles. In terms of toxicity, nanoparticles with or without MA were found to be acceptable for use, although MA did affect the viability of the cells more than poly, DL, lactic-co-glycolic acid particles alone in *in vitro* studies. This paves the way for testing MA as a ligand to target anti-TB drugs to the sites of infection in human TB patients.

Opsomming

Suid Afrika het tans die hoogste insidensie van TB per 100 000 mense in die wêreld. In 2007 het 112 000 mense gesterf van TB in Suid Afrika waarvan 94 000 mense ge-ko-infekteer was met MIV. Alhoewel TB behandeling bestaan, is daar ‘n groot uitdaging vir TB programme in die wêreld as gevolg van swak pasiënt-voldoening asook middel weerstandigheid wat ontstaan. Hierdie uitdaging is aangespreek deur die ontwikkeling van ‘n polimeriese anti-TB nanomiddel-aflewering stelsel wat opneembaarheid kan verhoog asook stadige vrystelling van die middel kan bewerkstellig wat dan die dosis kan verlaag en pasiënt-voldoening kan bevorder. Die doel van die projek was om die anti-TB nanomiddel aflewering stelsel te sintetiseer en dit na TB geïnfekteerde selle te teiken. Die nanopartikels was suksesvol gesintetiseer met geïnkorporeede anti-TB middels wat in selle opgeneem was. ‘n Moontlike teikenmiddel, mikoolsure van die *M. tuberculosis* bakterieë is ondersoek. Die mikoolsure kon geïnkorporeer word in die nanopartikels wat as ‘n ligand sou kon dien vir die cholesterol- ryke areas as gevolg van die cholesteroïede aard van die mikoolsure, asook die feit dat mikoolsure aangetrek word deur cholesterol. ‘n Ander moontlikheid is dat die mikoolsure met anti-mikoolsuur teenliggame kan reageer wat waarskynlik in hoër konsentrasies teenwoordig sal wees in die geïnfekteerde areas. Die cholesteroïede aard van die mikoolsure was bevestig, asook hoe die struktuur van die mikoolsure daarmee verband hou. Daar is aangetoon dat die mikoolsuur-bevattende nanopartikels *in vitro* in selle opgeneem word sonder dat die mikoolsure die opname belemmer het. In terme van toksisiteit was die nanopartikels met en sonder mikoolsure bevind om geskik te wees vir gebruik, alhoewel die mikoolsure die lewensvatbaarheid van die selle meer ge affekteer het as die poly, DL, lactic-co-glycolic acid-partikels alleen in *in vitro* studies. Die resultate maak die pad oop om mikoolsure te toets as ligande om anti-TB middels te teiken na die geïnfekteerde loki van TB pasiënte.