Biochemical characterisation of putrescine and spermidine uptake as a potential therapeutic target against the human malaria parasite, *Plasmodium falciparum*

by

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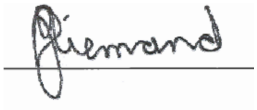
Signed: Jandeli Niemand 

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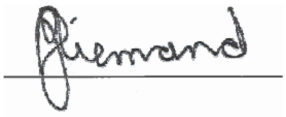
Signature:  Date: 24 August 2011

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Summary

Plasmodium falciparum causes the most severe form of human malaria, and the continual development of resistance of this parasite to current anti-malarial drugs underpins a pressing need for the discovery of novel chemotherapeutic approaches. Polyamines and their biosynthetic enzymes are present at high levels in rapidly proliferating cells, including cancer cells and protozoan parasites. Inhibition of the malaria parasite's polyamine biosynthesis pathway causes cytostatic arrest in the trophozoite stage, but does not cure infections *in vivo*. This may be due to the salvage of exogenous polyamines from the host, replenishing the intracellular polyamine pool; however the mechanism(s) of polyamine uptake by the intra-erythrocytic parasite are not well understood. In this study the uptake of the polyamines putrescine and spermidine into *P. falciparum*-infected erythrocytes (iRBC) well as into *P. falciparum* parasites functionally isolated from their host cell by saponin-permeabilisation of the erythrocyte membrane was investigated using radioisotope flux techniques. While the characteristics of transport of putrescine into infected erythrocytes were similar to those of transport into uninfected erythrocytes, spermidine entered iRBC in part via the 'new permeation pathways' induced by the parasite in the erythrocyte membrane. Both putrescine and spermidine were taken up across the plasma membrane of isolated parasites via a saturable, temperature-dependent process that showed competition between different polyamines as well as the polyamine precursor ornithine and basic amino acids. Inhibition of polyamine biosynthesis led to increased total uptake of both putrescine and spermidine. The influx of putrescine and spermidine into isolated parasites was independent of Na^+ but increased with increasing pH and showed a marked dependence on the membrane potential, decreasing with membrane depolarisation and increasing with membrane hyperpolarisation.

Both anthracene and polyamine derivatives have been shown to have anti-malarial activity. Anthracene-polyamine conjugates have been developed with the aim of utilising the polyamine uptake mechanisms of cancer cells to deliver the cytotoxic anthracene moieties to these cells. Here, several anthracene-polyamine conjugates showed promising anti-malarial activity. These compounds inhibited parasite proliferation with IC_{50} values in the nM range, and caused an arrest in the cell cycle, as well as a decrease in the mitochondrial membrane potential. Cytotoxicity could not be reversed by the addition of exogenous polyamines, nor did the conjugates have an effect on intracellular polyamine levels.

This doctoral study showed that *P. falciparum* parasites not only synthesise polyamines, but can also acquire putrescine and spermidine from the extracellular environment and paves the way for interfering with polyamine metabolism as an anti-parasitic strategy.

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$\Delta\psi$	Membrane potential
$\Delta\psi$m	Mitochondrial membrane potential
AdoMet	*S*-adenosylmethionine
AdoMetDC	*S*-adenosylmethionine decarboxylase
AMEL-3	Hamster melanoma cell line
APA	3-aminooxy-1-aminopropane
APAO	N^1 acetylpolyamine oxidase
APC	Amino acid/Polyamine/Organocation
BCBD	N^1,N^4-bis-(7-chloroquinoline-4-yl)butane-1,4-diamine
BCECF	2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein
BP	Bandpass
CCC	Cation-Cl^- cotransporter
CCCP	Carbonyl cyanide-*m*-chlorophenylhydrazone
CHO	Chinese hamster ovary
DAX	Diamine exporter
dcAdoMet	Decarboxylated *S*-adenosylmethionine
DCFDA	2'-7'-Dichlorodihydrofluorescein diacetate
DFMO	DL-α-difluoromethylornithine
DHFR	Dihydrofolate reductase
eIF5A	Eukaryotic initiation factor 5A
EPM	RBC plasma membrane
FACS	Fluorescence-activated cell sorting
Gpc-1	Glypican-1
HEK-293	Human embryonic kidney 293 cells
HEPES	*N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulphonic acid)
HL-60	Human leukaemia cell line
iRBC	*P. falciparum* (strain 3D7)-infected red blood cell
iRBCs	*P. falciparum* (strain 3D7)-infected red blood cells
JC-1	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide
L1210	Murine leukaemia cells
MDL27695	*N,N*-bis{3-(phenylmethyl)aminolpropyl}-1,7-diaminoheptane
MES	2-morpholinoethanesulfonic acid

MGBG	Methylglyoxal bis(guanylhydrazone)
MMV	Medicines for Malaria Venture
MR	Methionine recycling pathway
MTA	5'methylthioadenosine
NMDG	*N*-methyl-D-glucamine
NO	Nitric oxide
NOS2	Nitric oxide synthase
NPP	New Permeation Pathways
ODC	Ornithine decarboxylase
PAH	Polycyclic aromatic hydrocarbons
PAO	Polyamine oxidase
PBS	Phosphate-buffered saline
PfAdoMetDC/ODC	*P. falciparum S*-adenosylmethionine decarboxylase/ornithine decarboxylase
PfATP4	*P. falciparum* Ca^{2+}ATPase
PfCHA	Putative *P. falciparum* Ca^{2+}/H^{+} anti-porter
PfCRT	*P. falciparum* chloroquine-resistance transporter
PfENT1	*P. falciparum* Equilibrative Nucleoside/nucleobase Transporter 1
pH_i	Intracellular pH
pH_o	Extracellular pH
PPM	Parasite plasma membrane
PSAC	Plasmodial surface anion channel
PVM	Parasitophorous vacuolar membrane
RBC	Uninfected human red blood cell
RBCs	Uninfected human red blood cells
ROS	Reactive oxygen species
rpm	Revolutions per minute
S.E.	Standard error of the mean
SAM3	*S*-Adenosylmethionine transporter
SAMI	South African Malaria Initiative
-SH	Sulfhydryl
SI	Selectivity index
SMO	Spermine oxidase
SMR	Small multi-drug resistance

SpdSyn	Spermidine synthase
SpmSyn	Spermine synthase
Spp	Species
SSAT:	Spermidine/spermine N^1-acyltransferase
TPO	Transporter for polyamine
TS	Thymidylate synthethase
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick end-labelling
WHO	World health organisation