THE ANTIPLASMODIAL ACTIVITIES OF THE TETRAMETHYLPIPERIDYL-SUBSTITUTED PHENAZINES, B4119 AND B4158

by

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To the memory of my beloved mother

NGWANABOTLOU MONICA CHUENE

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SUMMARY

A novel flow cytometric procedure was established for use in evaluating the *in vitro* antimalarial activity of tetramethylpiperidine (TMP)- substituted phenazines. The flow cytometric procedure was compared with microscopy and radiometry for efficiency in quantitating the level of parasitemia in malaria cultures. The flow cytometric method compared well, as determined by the Bland and Altman measure of agreement, with both microscopy and radiometry and was chosen for use in this study due to its speed, precision and convenience (includes a fixing step that allows samples to be evaluated at any one time). The TMP-substituted phenazines B4119 and B4158, synthetic derivatives of clofazimine, were evaluated extensively against a drug-sensitive and various drug-resistant lines of *Plasmodium falciparum in vitro* and against *P. berghei* in mice. Parasite growth was measured using microscopic and flow cytometric methods, while heme polymerization was investigated using an infrared spectroscopic procedure. The therapeutic potential of B4119 alone (30mg/kg/day), and in combination with a sub-therapeutic dose of chloroquine (1.25µg/kg/day) was measured in a murine model of experimental infection with *P. berghei*.

B4119 and B4158, but not clofazimine, inhibited the growth of the drug-sensitive strain of *P. falciparum* with respective IC_{50} values of 0.22µM and 0.4µM, while the drug-resistant strains of the parasite were equally sensitive to the TMP-substituted phenazines, indicating a lack of cross-resistance. Augmentation of anti-plasmodial activity was observed when B4119 and B4158 were used in combination with chloroquine or mefloquine. The compounds were capable of inhibiting all blood stages of *P. falciparum*. Pretreatment of erythrocytes with B4119 and B4158 did not prevent merozoite invasion. B4119- and B4158-mediated inhibition of the growth of *P. falciparum* was associated with interference with heme polymerisation to β-haematin *in vitro*. Administration of B4119 to *P. berghei*-infected mice was accompanied by a significant reduction in parasitemia, while additive therapeutic activity was observed when this agent was combined with chloroquine.

The TMP-substituted phenazines B4119 and B4158 are promising, novel anti-plasmodial agents.

OPSOMMING

'n Nuwe vloeisitometriese prosedure is ontwikkel om te gebruik in die evaluering van die *in vitro* antimalaria aktiwiteit van tetrametielpiperidien (TMP)-gesubstitueerde fenasiene. Die effektiwiteit van die vloeisitometriese prosedure om die vlakke van parasitemie in malaria kulture te bepaal is met die mikroskopiese en radiometriese metodes vergelyk. Die vloeisitometriese metode het, soos bepaal deur die Bland en Altman se mate van ooreenstemming, goed met beide die mikroskopiese en radiometriese metodes vergelyk en is vir hierdie studie gekies aangesien dit vinnig, akkuraat en gerieflik is. Hierdie metode het 'n fikseringsstap ingesluit wat dit moontlik gemaak het om die monsters op 'n latere geleentheid te evalueer. Die TMP-gesubstitueerede fenasiene B4119 en B4158, sintetiese derivate van klofasimien, is breedvoerig teen 'n geneesmiddel-sensitiewe en verskeie geneesmiddel-bestande lyne van *Plasmodium falciparum in vitro* en teen *P. berghei* in muise ondersoek. Parasietgroei is deur middel van mikroskopiese en vloeisitometriese metodes bepaal terwyl heem-polimerisasie ondersoek is deur die gebruik van spektroskopiese prosedures. Die terapeutiese potential van B4119 alleen (30mg/kg'dag) en in kombinasie met 'n sub-terapeutiese dosis van chlorokien (1.25µg/kg/dag) is in 'n muis model van eksperimetele infeksie met *P. berghei* bepaal.

B4119 en B4158, maar nie klofasimien, het die groei van die geneesmiddel-sensitiewe stam van *P. falciparum* by IK_{50} waardes van 0.22µM en 0.4µM respektiewelik geïnhibeer, terwyl die geneesmiddel-bestande stamme van die parasiet ewe sensitief was vir die TMP-gesubstitueerde fenasiene, wat op die afwesigheid van kruis-bestandheid dui. Verhoging van anti-plasmodiale aktiwiteit is waargeneem wanneer B4119 en B4158 in kombinasie met chlorokien en meflokien gebruik is. Die verbindings was in staat om alle bloed-stadiums van *P. falciparum* te inhibeer vooraf behandeling van eritrosiete met B4119 en B4158 het nie die indring van merozïete verhoed nie. B4119- en B4158-bemiddelde inhibisie van die groei van *P. falciparum* is met veranderinge in heem polimerisasie tot β-hematien *in vitro* geassosieer. Die toediening van B4119 aan *P. berghei*-geïnfekteerde muise het tot 'n betekenisvolle vermindering in parasitemie gelei, terwyl 'n vermeerdering in terapeutiese aktiwiteit waargeneem is Indians die verbinding met chlorokien gekombineer is. Die TMP-gesubstitueerde fenasiene B4119 en B4158 is belowende, nuwe anti-plasmodiale middels.

LIST OF ABBREVIATIONS

ADCI	Antibody-dependent cellular immunity
AIDS	Acquired immunodeficiency syndrome
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BBIQ	Bisbenzylisoquinolines
Ca	Calcium
cAMP	Cyclic adenosine monophosphate
CD	Cluster of differentiation
CO_2	Carbon dioxide
CQ	Chloroquine
CQR	Chloroquine resistant
CQS	Chloroquine sensitive
CSA	Chondroitin sulphate A
DHFR	Dihydrofolatereductase
DDT	Dichloro-diethyl-trichloroethane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EBA	Erythrocyte binding antigen
EIPA	5-(N-ethyl-N-isopropyl) amiloride
ELISA	Enzyme-linked immunosorbent assay
FCS	Fetal calf serum
G3PDH	Glyceraldehyde 3-phosphate dehydrogenase
H	Hydrogen
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HPIA	Heme polymerization inhibitory activity
ICAM-1	Intercellular adhesion molecule-1
iRBC	Infected red blood cell
K	Potassium
MDR	Multidrug resistance
Mef	Mefloquine

MRC	**Medical Research Council**
MSP-1	**Merozoite surface protein-1**
Na	**Sodium**
NaCl	**Sodium chloride**
NAD	**Nicotinamide dinucleotide**
NADPH	**Nicotinamide adenine dinucleotide phosphate (reduced)**
NHE	**Na+/H+ exchanger**
NMRP	**National Malaria Research Programme**
NPPB	**5-nitro-2-(3-phenylpropylamino) benzoic acid**
NRA	**Nucleoside releasing agent**
O_2	**Oxygen**
PABA	**Para-aminobenzoic acid**
PBS	**Phosphate buffered saline**
PCT	**Parasite clearance time**
***PF*EMP-1**	***Plasmodium falciparum* erythrocyte membrane protein-1**
***PF*EMP-2**	***Plasmodium falciparum* erythrocyte membrane protein-2**
***PF*HRP-1**	***Plasmodium falciparum* histidine rich protein-1**
PG	**Prostaglandin**
PGE_2	**Prostaglandin E_2**
Pgh1	**P-glycoprotein homologue-1**
PKC	**Protein kinase C**
PLA_2	**Phospholipase A_2**
PRR	**Parasite reduction ratio**
PSD	**Sulfadoxine/pyrimethamine**
PVM	**Parasitophorous vacuolar membrane**
Rb	**Rubidium**
RBC	**Red blood cell**
RESA	**Ring-infected erythrocyte surface antigen**
RNA	**Ribonucleic acid**
TCA	**Tricarboxylic acid**
TMP	**Tetramethylpiperidine**
TSP	**Thrombospondin**

VCAM-1	**Vascular adhesion molecule-1**
WHO	**World Health Organization**

LIST OF FIGURES

Figure 42 : Effects of intra peritoneal administration of chloroquine (0.25µg/kg/day) together with oral administration of B4119 (30mg/kg/day) on the survival rate of *P. berghei*-infected Balb/C mice.

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