

# CONTENTS

	Page
Acknowledgement	iii
Abstract in Thai	v
Abstract in English	vii
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xv
Chapter 1 Introduction	1
1.1 Statement and significant of the problems	1
1.2 Literature reviews	3
1.2.1 Incidence of malaria infection	3
1.2.2 Life cycle of malaria parasite	4
1.2.3 Anti-folate drugs used in malaria	7
1.2.4 Iron chemistry and chelator	12
1) Desferrioxamine (DFO)	13
2) Deferiprone (DFP)	14
3) Deferasirox (DFX)	15
4) CM1	15
1.2.5 Green tea	16
1.3 Objectives of the study	18
Chapter 2 Materials and Methods	19
2.1 Chemicals and reagents	19
2.2 Materials	19
2.2.1 Test compounds	19
1) Antimalarial drugs	19

2) Chelators	19
3) 1-( <i>N</i> -Acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one, a hydroxypyridin-4-one (CM1)	19
4) Green tea extract (GTE)	20
2.2.2 Parasite strains	20
1) <i>P. falciparum</i> strain 3D7	20
2) <i>P. berghei</i> strain ANKA expressing GFP	21
2.2.3 Mice	21
2.3 Methods	21
2.3.1 <i>In vitro P. falciparum</i> culture	21
1) Malaria culture medium preparation	21
2) Red blood cells (RBC) preparation for malaria culture	22
3) Culturing of asexual erythrocytic stages of <i>P. falciparum</i> <i>in vitro</i> culture in culture Petri dish	23
4) Sorbitol synchronization of <i>P. falciparum</i> -infected RBC	24
5) Cryopreservation of <i>P. falciparum</i> blood stage parasites	25
6) Thawing of glycerolyte-frozen parasites with NaCl	25
2.3.2 <i>In vitro</i> drug-susceptibility testing of <i>P. falciparum</i>	26
1) Single drug treatment	26
2) Drug combination treatment	27
2.3.3 Determination of erythrocytic stages of <i>P. falciparum</i> parasite by fluorescent flow cytometric assay	28
2.3.4 Measurement of LIP in <i>P. falciparum</i> -infected RBC	29
2.3.5 Detection of ROS in <i>P. falciparum</i> -infected RBC	31
2.3.6 Manipulation of rodent malaria parasite <i>P. berghei in vivo</i>	33
1) Cryopreservation of <i>P. berghei</i> blood stage parasites	34
2) Malarial infection of laboratory animal	34
2.3.7 <i>In vivo</i> drug-susceptibility testing of <i>P. berghei</i>	34
1) Single drug treatment	34
2) Drug combination treatment	36
2.4 Statistical analysis	36

## CONTENTS (CONTINUED)

	Page
Chapter 3 Results	37
3.1 Flow cytometric analysis of <i>P. falciparum</i> parasite	37
3.2 <i>In vitro</i> drug-susceptibility testing of <i>P. falciparum</i>	40
3.2.1 Single drug treatment	40
3.2.2 Drug combination treatment	43
3.3 Effect of iron chelators and GTE on intracellular LIP levels in <i>P. falciparum</i> -infected RBC	46
3.4 Effect of chelators and GTE on intracellular ROS levels in <i>P. falciparum</i> -infected RBC	51
3.5 <i>In vivo</i> drug-susceptibility testing of <i>P. berghei</i>	55
3.5.1 Single drug treatment	55
3.5.2 Drug combination treatment	58
Chapter 4 Discussion and Conclusion	60
References	64
Appendices	76
Appendix A	77
Appendix B	78
Appendix C	80
Appendix D	83
Curriculum Vitae	95

## LIST OF TABLES

	Page
Table 3-1 <i>In vitro</i> susceptibilities of <i>P. falciparum</i> and iron chelators and GTE	40
Table 3-2 <i>In vitro</i> susceptibilities of <i>P. falciparum</i> and antimalarial drugs	41
Table S-1 Effect of DFO on growth of <i>P. falciparum</i>	83
Table S-2 Effect of DFP on growth of <i>P. falciparum</i>	83
Table S-3 Effect of CM1 on growth of <i>P. falciparum</i>	84
Table S-4 Effect of GTE on growth of <i>P. falciparum</i>	84
Table S-5 Effect of DFX on growth of <i>P. falciparum</i>	85
Table S-6 Effect of PYR on growth of <i>P. falciparum</i>	85
Table S-7 Effect of DHA on growth of <i>P. falciparum</i>	86
Table S-8 Effect of PYR combined with CM1 on growth of <i>P. falciparum</i>	87
Table S-9 Effect of PYR combined with GTE on growth of <i>P. falciparum</i>	87
Table S-10 Levels of LIP in <i>P. falciparum</i> -infected RBC treated with CM1 (0-200 $\mu$ M)	88
Table S-11 Levels of LIP in <i>P. falciparum</i> -infected RBC treated with DFP (0-200 $\mu$ M)	88
Table S-12 Levels of LIP in <i>P. falciparum</i> -infected RBC treated with GTE (0-200 $\mu$ M)	88
Table S-13 Levels of ROS in <i>P. falciparum</i> -infected RBC treated with CM1 (0-200 $\mu$ M)	89
Table S-14 Levels of ROS in <i>P. falciparum</i> -infected RBC treated with DFP (0-200 $\mu$ M)	89
Table S-15 Levels of ROS in <i>P. falciparum</i> -infected RBC treated with GTE (0-200 $\mu$ M)	89

## LIST OF TABLES (CONTINUED)

	Page
Table S-16 Effect of PYR on <i>P. berghei</i> growth in infected mice	90
Table S-17 Effect of CM1 on <i>P. berghei</i> growth in infected mice	90
Table S-18 Effect of GTE on <i>P. berghei</i> growth in infected mice	90
Table S-19 Effect of PYR combined with CM1 on <i>P. berghei</i> growth in infected mice	91
Table S-20 Effect of PYR combined with GTE on <i>P. berghei</i> growth in infected mice	91
Table S-21 The percentage of parasite growth after treatment with CM1 for 2 h, in experiment of measurement of LIP in <i>P. falciparum</i> -infected RBC	92
Table S-21 The percentage of parasite growth after treatment with DFP for 2 h, in experiment of measurement of LIP in <i>P. falciparum</i> -infected RBC	92
Table S-21 The percentage of parasite growth after treatment with GTE for 2 h, in experiment of measurement of LIP in <i>P. falciparum</i> -infected RBC	92

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

## LIST OF FIGURES

	Page
Figure 1-1 Life cycle of malaria parasite	6
Figure 1-2 Pathway of folate biosynthesis in <i>Plasmodium</i> spp.	8
Figure 1-3 Chemical structures of pyrimethamine, cycloguanil, proguanil, chlorproguanil	10
Figure 1-4 Chemical structures of artemisinin and its derivatives	11
Figure 1-5 Chemical structures of DFO, DFP, DFX and CM1	13
Figure 1-6 Chemical structures of catechins in green tea ( <i>Camellia sinensis</i> )	17
Figure 2-1 The principle of LIP	29
Figure 2-2 Principle of DCF assay	32
Figure 3-1 Analysis of SYBR Green I-fluorescence intensity by flow cytometry of <i>P. falciparum</i> -infected RBC	38
Figure 3-2 Correlation of flow cytometric and microscopic methods for determination of parasitemia	39
Figure 3-3 Effect of iron chelators and GTE on growth of <i>P. falciparum</i>	41
Figure 3-4 Effect of antimalarial drugs PYR and DHA on growth of <i>P. falciparum</i>	42
Figure 3-5 Effect of PYR combined with CM1 on growth of <i>P. falciparum</i>	44
Figure 3-6 Effect of PYR combined with GTE on parasite growth of <i>P. falciparum</i>	45
Figure 3-7 Discrimination of PRBC from NRBC by flow cytometry using SYTO 61 dye	46
Figure 3-8 Measurement of intracellular LIP in <i>P. falciparum</i> -infected RBC using flow cytometry	47

## LIST OF FIGURES (CONTINUED)

	Page
Figure 3-9 Levels of LIP in <i>P. falciparum</i> -infected RBC treated with CM1 (0-200 $\mu$ M) for 2 h	48
Figure 3-10 Levels of LIP in <i>P. falciparum</i> -infected RBC treated with DFP (0-200 $\mu$ M) for 2 h	49
Figure 3-11 Levels of LIP in <i>P. falciparum</i> -infected RBC treated with GTE (0-200 $\mu$ M EGCG equivalent) for 2 h	50
Figure 3-12 Levels of ROS in <i>P. falciparum</i> -infected RBC treated with CM1 (0-200 $\mu$ M) for 2 h	52
Figure 3-13 Levels of ROS in <i>P. falciparum</i> -infected RBC treated with DFP (0-200 $\mu$ M) for 2 h	53
Figure 3-14 Levels of ROS in <i>P. falciparum</i> -infected RBC treated with GTE (0-200 $\mu$ M EGCG equivalent) for 2 h	54
Figure 3-15 Effect of PYR on <i>P. berghei</i> growth in infected mice	55
Figure 3-16 Effect of CM1 on <i>P. berghei</i> growth in infected mice	56
Figure 3-17 Effect of GTE on <i>P. berghei</i> growth in infected mice	57
Figure 3-18 Effect of PYR combined with CM1 on <i>P. berghei</i> growth in infected mice	58
Figure 3-19 Effect of PYR combined with GTE on <i>P. berghei</i> growth in infected mice	59
Figure S-1 The percentage of parasite growth after treatment with CM1 for 2 h, in experiment of measurement of LIP in <i>P. falciparum</i> -infected RBC	92
Figure S-2 The percentage of parasite growth after treatment with DFP for 2 h, in experiment of measurement of LIP in <i>P. falciparum</i> -infected RBC	93
Figure S-3 The percentage of parasite growth after treatment with GTE for 2 h, in experiment of measurement of LIP in <i>P. falciparum</i> -infected RBC	94

## LIST OF ABBREVIATIONS

%	Percent
μl	Microlitre
μm	Micrometre
μM	Micromolar
°C	Degree Celsius
CA-AM	Calcein acetoxymethyl ester
CI	Confidence interval
CM1	1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one, hydroxypyridin-4-one derivative
CQ	Chloroquine
Cyc	Cycloguanil
DCF	Dichlorofluorescein
DCFH-DA	2',7'-Dichlorofluorescein diacetate
DFO	Desferrioxamine
DFP	Deferiprone, 1,2-dimethyl-3-hydroxypyrid-4-one, L1
DFX	Deferasirox, ICL670
DHA	Dihydroartemisinin
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase enzyme
DHPS	Dihydropteroate synthase enzyme
dl	Decilitre
DMSO	Dimethyl sulfoxide
ED <sub>50</sub>	Effective dose at 50%
EGCG	(-)-Epigallocatechin 3-gallate
Fe	Iron
FI	Fluorescent intensity
FITC	Fluorescein isothiocyanate
g	Gavity force
g	Gram



GFP	Green Fluorescent Protein
GTE	Green tea extract
h	Hour
i.p.	Intraperitoneal route
IC <sub>50</sub>	Inhibitory concentration at 50%
K <sub>a</sub>	Association constant
kg	Kilogram
L	Litre
LIP	Labile iron pool
LPI	Labile plasma iron
M	Molar
MDA	Malondialdehyde
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
mW	Milliwatt
NaCl	Sodium chloride
nm	Nanometre
nM	Nanomolar
NRBC	non-infected red blood cell
NTBI	Non-transferrin-bound serum iron
<i>P. berghei</i>	<i>Plasmodium berghei</i>
<i>P. falciparum</i>	<i>Plasmodium falciparum</i>
<i>P. knowlesi</i>	<i>Plasmodium knowlesi</i>
<i>P. malariae</i>	<i>Plasmodium malariae</i>
<i>P. ovale</i>	<i>Plasmodium ovale</i>
<i>P. vivax</i>	<i>Plasmodium vivax</i>
<i>P. yoelii</i>	<i>Plasmodium yoelii</i>
PBMC	Peripheral blood mononuclear cells

PBS	Phosphate-buffered saline
PE	Phycoerythrin
pfdhfr	<i>Plasmodium falciparum</i> dihydrofolate reductase gene
pfdhps	<i>Plasmodium falciparum</i> dihydropteroate synthase gene
PRBC	Parasitized red blood cell, parasite-infected red blood cell
PYR	Pyrimethamine
RBC	Red blood cell
ROS	Reactive oxygen species
SDX	Sulfadoxine
sec	Second
THF	Tetrahydrofolate
v/v	Volume by volume
w/v	Weight by volume
w/w	Weight by weight



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright© by Chiang Mai University  
 All rights reserved