

FORMULATION OF QUININE
SUPPOSITORY AND EVALUATION OF
QUININE UPTAKE IN THE MOUSE
BRAIN

BY

SOREMEKUN REBECCA ORITSEMAJE

NOVEMBER 2010

FORMULATION OF QUININE
SUPPOSITORY AND EVALUATION OF
QUININE UPTAKE IN THE MOUSE
BRAIN

A THESIS SUBMITTED TO THE UNIVERSITY OF
LAGOS, NIGERIA

IN PARTIAL FULFILLMENT OF THE
REQUIREMENT FOR THE AWARD OF DOCTOR OF
PHILOSOPHY (Ph.D.) IN CLINICAL PHARMACY

BY

SOREMEKUN REBECCA ORITSEMAJE

NOVEMBER 2010

SCHOOL OF POSTGRADUATE STUDIES
UNIVERSITY OF LAGOS

CERTIFICATION

This is to certify that the Thesis:

“FORMULATION OF QUININE SUPPOSITORY AND
EVALUATION OF QUININE UPTAKE IN THE MOUSE BRAIN”

Submitted to the
School of Postgraduate Studies
University of Lagos

For the award of the degree of
DOCTOR OF PHILOSOPHY (Ph.D.)
is a record of original research carried out
By

SOREMEKUN, REBECCA ORITSEMAJE
In the Department of Clinical Pharmacy and Biopharmacy

----- AUTHOR'S NAME	----- SIGNATURE	----- DATE
----- 1 ST SUPERVISOR'S NAME	----- SIGNATURE	----- DATE
----- 2 ND SUPERVISOR'S NAME	----- SIGNATURE	----- DATE
----- 1 ST INTERNAL EXAMINER	----- SIGNATURE	----- DATE
----- 2 ND INTERNAL EXAMINER	----- SIGNATURE	----- DATE
----- EXTERNAL EXAMINER	----- SIGNATURE	----- DATE
----- SPGS REPRESENTATIVE	----- SIGNATURE	----- DATE

DEDICATION

I dedicate this work to my husband, Olukayode Akinyemi Soremekun for his love and unflinching support as regards the progress and development of my career. My daughters: Olumayokun, Yemisi, Eniola, Ayokunmi , Oluwatoowo and Opemipo have a pride of place in my life and by extension in the completion of this work.

ACKNOWLEDGEMENTS

I thank the almighty God for His hands that have been at work in my life. It was Him that put a detour in my career path such that I could not embark on the Ph.D. route until now. He is the mighty artist who has the full picture. I appreciate Him daily even as the picture emerges.

I also wish to place on record my sincere appreciation of my supervisor: Prof Fola Tayo. Professor Tayo's avuncular dispositions are such that his thoroughness and cerebral inputs course through the work. For all these and more, I sincerely appreciate you sir. I also wish to appreciate my second supervisor, Prof Cecilia Igwilo. Her initial and continuous encouragement inspired me to pursue an academic career. My sincere appreciation also goes to Dr Lanre Silva whose tremendous encouragement helped me to struggle on through the tough times. Dr Silva was more like a third supervisor. I must also appreciate the dedication of Mr Ojobo, the technologist at the Central Research laboratory of the College of Medicine. He is an epitome of diligence and I have no doubt that he will attain great heights. I also wish to appreciate the Dean of the Faculty, Prof HAB Coker for his concern and constant advice. Prof Omilabu also deserves mention. He made out time to put me through on the use of the fluorescent microscope and made same available for my use. Mr Seyi Campbell who provided assistance with the light microscopy also deserves my gratitude. I also wish to appreciate the technical staff of the Department of Clinical Pharmacy especially; Mrs Adams, Mrs Abbey and David. They were most supportive in the course of this work. To all my colleagues in the Department, permit me to say that; you make the environment more conducive and interesting. Thank you very much.

Lastly, I appreciate the love and prayers of my biological and spiritual siblings. I pray that my father, the Almighty God will reward you all beyond measure. God bless you all.

TABLE OF CONTENTS

LEGEND	PAGES
TITLE PAGE	i
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v
LIST OF TABLES	xi
LIST OF FIGURES	xii
APPENDIX	xiv
ABSTRACT	xv

CHAPTER ONE

1.0.	INTRODUCTION	1
1.1	STATEMENT OF THE PROBLEM	1
1.2	OBJECTIVES OF THE STUDY	3
1.3	SIGNIFICANCE OF STUDY	3
1.4	LIMITATIONS OF STUDY	3
1.5	RESEARCH QUESTIONS	4
1.6	OPERATIONAL DEFINITION OF TERMS/ABBREVIATIONS	4

CHAPTER TWO

2.0.	LITERATURE REVIEW	5
2.1	MALARIA,	5
2.1.1	Overview of malaria	5
2.1.2	Malaria Situation in Nigeria	10
2.1.3	Economic Burden	11
2.1.4	Malaria Control Policy and Strategy	12
2.1.5	Roll Back Malaria	13
2.2	SEVERE AND COMPLICATED MALARIA AND TREATMENT	17
2.2.1	Severe and Complicated Malaria	17
2.2.2	Cerebral Malaria	18
2.2.2.1	Pathophysiology	19
2.2.2.2	Murine Mice Model	24
2.2.3	Chemotherapy of Cerebral malaria	25
2.3.	QUININE	28
2.3.1.	Quinine Monograph	28
2.3.2.	Quinine Bisulphate	28
2.3.3.	Definition and Description	29
2.3.4.	Pharmacology and Toxicology	29
2.3.5.	Uses and Therapeutic Dosage	32
2.3.6.	Clinical Toxicity	34

2.3.7.	Chronic Poisoning	34
2.3.8.	Management of Over-Dosage and Toxicity	37
2.3.9.	Mechanism of Action and Pharmacokinetics	37
2.4	THE SUPPOSITORY AS A DOSAGE FORM	42
2.4.1	The Suppository	42
2.4.2	Suppository Bases	44
2.4.3	Drug substance related factors	44
2.4.4	Surfactants	46
2.4.4.1	Classification of Surfactants	47
2.4.4.1.1	Non-Ionic Surfactants	47
2.4.4.1.2.	Anionic Surfactants	48
2.4.4.1.3	Cationic Surfactants	48
2.4.4.1.4	Amphoteric Surfactants	49
2.5	THE RECTAL ROUTE	50
2.5.1	Anatomy and Physiology of the Rectal Route	50
2.5.2	Absorption of Drugs from the Rectum	50
2.5.3	Antimalarials in Suppository Dosage Form	53
2.6	DISSOLUTION STUDIES	56
2.6.1	Theory of Dissolution	56
2.6.1	In-vitro to In-vivo Correlation	57
2.7.	BRAIN COMPONENTS AND FUNCTIONS	59
2.7.1	Olfactory lobe	59

2.7.2	Cerebrum	59
2.7.3	Cerebellum	59
2.7.4	Brain Stem	60
2.7.5	Blood brain barrier	61

CHAPTER THREE

3.	MATERIALS AND METHOD	64
3.1	MATERIALS AND EQUIPMENT	64
3.1.1	Materials	64
3.1.2	Equipment	64
3.2	PRE-FORMULATION STUDIES	65
3.2.1	Choice of Base	65
3.2.2	Particle Size Determination	66
3.2.3	Characterization and Assays	66
3.2.3.1	Characterization	66
3.2.3.2	Assay	66
3.2.4	Determination of Displacement Value	67
3.2.5	Determination of Quinine Dose in Suppository	68
3.3	FORMULATION STUDIES	68
3.3.1	Preparation of Suppository	68
3.3.2	Characterization of Prepared Suppositories	69
3.4	RELEASE PROFILE AND STABILITY STUDIES	71

3.5	ANIMAL STUDIES	72
3.5.1	Preliminary Quality Evaluation of Quinine Fluorescence in water	72
3.5.2.	Monitoring of <i>Plasmodium berghei</i> in the brain of mice	72
3.5.3	Location of Quinine Fluorescence in Brain Sections	72
3.5.4	Uptake of Quinine from Suppository Formulation in the Brain Sections	73
3.5.5	Uptake of Quinine from Intra-peritoneal Administration of Quinine	75
3.5.6	Quantification of Quinine in Brain Sections	75
3.5.6.1	Method of Extraction	75
3.5.6.2	Determination of Standard Curve	76
3.5.6.3	Determination of Extraction Efficiency	76
 CHAPTER FOUR		
4.0	RESULTS	78
4.1	PRE-FORMULATION STUDIES	78
4.1.1	Particle size determination	78
4.1.2	Characterization of bases	78
4.1.3	Displacement value in bases	78
4.1.4	Assay of active ingredients and excipients	79
4.2	FORMULATION STUDIES	79
4.2.1	Characterization of suppositories	79
4.3	RELEASE PROFILE OF QUININE FROM SUPPOSITORIES	81

4.4	STABILITY STUDIES	84
4.5	ANIMAL STUDIES	88
4.5.1	Quinine fluorescence in brain cells	89
4.5.2	Parasites in four brain sections	90
4.5.3	Quantitative measurements of quinine in brain parts	91
4.5.4	Quinine uptake in parasitized and non-parasitized brain sections of mice	93
4.5.5	Comparative Uptake of Quinine in Brain Sections	95
4.5.6	Uptake in Whole Brain	102
4.5.7	Uptake from Intra-peritoneal Injection	103

CHAPTER FIVE

5.0	DISCUSSION	104
------------	-------------------	------------

CHAPTER SIX

6.0	CONCLUSION AND CONTRIBUTIONS TO KNOWLEDGE	119
6.1	Conclusion	119
6.2	Contributions to knowledge	120

REFERENCES	121
-------------------	------------

APPENDIX I	146
-------------------	------------

APPENDIX II	148
--------------------	------------

LIST OF TABLES

Table

1	Intervention to control malaria and principal goals	16
2	Release characteristics of drugs in various suppository bases	45
3	Formula for prepared suppositories	69
4	Iodine value, acid value, saponification value and melting point of bases	78
5	Displacement value of quinine and polysorbate 80 in cocoa butter and Fattibase	78
6	Weight variation of suppositories	80
7	Percentage quinine content in suppository	80
8	Hardness of suppositories	81
9	Mean percentage released from refrigerated samples	85
10	Efficiency of extraction method	92
11	Between Factor Group Estimates	98
12	Between Factor Time Estimates	98
13	Comparative total uptake of quinine from suppository and intra-peritoneal injection	103
14	Concentration of extracted quinine from spiked brain tissue	146
15	Total Quinine Uptake in Whole Brain of Parasitized And Non-Parasitized Mice	147
16	Mean and standard for quinine release profile from formula H	148
17	Tests of Within-Subjects Effects	149
18	Mauchly's Test of Sphericity	150
19	Between Factor Group Estimates	151
20	Univariate Tests	151

21	Between Factor Time Estimates	152
22	Univariate test	152
23	Tests of Within-Subjects Effects	153
24	Tests of Between-Subjects Effects	154
25	Group Pairwise comparison	155
26	Time- Pairwise comparison	155
27	Brain sections – Pairwise comparison	156

LIST OF FIGURES

Figure		Page
1	Life Cycle of human malaria	5
2	Urban-rural difference differentials in <5s with fever, coverage with mosquito nets and those with antimalaria treatment	9
3	Estimated fever and malaria incidence in children < 5 years	10
4	Quinine bisulphate (9-hydroxy-6-methoxycinchonan sulphate heptahydrate	28
5	Structure of a surfactant molecule	47
6	Amount of quinine released from cocoa butter suppositories	82
7	Amount of quinine released from Fattibase TM Suppositories	83
8	Release profile of quinine in Fattibase TM + 5% polysorbate 80 stored in ambient temperature	85
9	Release profile of quinine in Fattibase TM + 5% polysorbate 80 stored in refrigerator	85

10	Comparison of release profile of samples stored in refrigerator and ambient temperature	86
11	Photomicrograph of healthy brain tissue of mice	89
12	Photomicrograph of healthy brain tissue of mice with quinine	89
13	Photomicrograph of parasitized brain without quinine	89
14	Photomicrograph of parasitized mice brain with quinine	89
15	Photomicrograph of parasites in olfactory lobe	90
16	Photomicrograph of parasites in cerebrum	90
17	Photomicrographs of parasites in cerebellum	90
18	Photomicrographs of parasites in medulla oblongata	90
19	Concentration of spiked quinine vs area of absorption	91
20	Quinine uptake in olfactory lobe of parasitized and non-parasitized mice	93
21	Quinine uptake in cerebrum of parasitized and non-parasitized mice	93
22	Quinine uptake in cerebellum of parasitized and non-parasitized mice	94
23	Quinine uptake in medulla oblongata of parasitized and non-parasitized	94
	Mice	
24	Comparative uptake in brain sections of parasitized and non-parasitized mice in 30 min	95
25	Comparative uptake in brain sections of parasitized and non-parasitized mice in 60 min	96
26	Comparative uptake in brain sections of parasitized and non-parasitized mice in 120 min	96
27	Comparative uptake in brain sections of parasitized and non-parasitized Mice in 180 min	97
28	Comparative uptake in brain sections of parasitized and non-parasitized	97

	mice in 240 min	
29	Estimated marginal means of concentrations vs time for brain sections	100
30	Estimated marginal means of concentrations versus time for parasitized and non-parasitized mice	101
31	Quinine uptake per mg of whole brain of mice	102
32	Standard curve of neat quinine sample	146

APPENDIX

Appendix 1

1.1.	Standard curve of neat quinine sample	146
1.2.	Concentration of extracted quinine from spiked brain tissue	146
1.3.	Total Quinine Uptake in Whole Brain of Parasitized And Non-Parasitized Mice	147

Appendix 2

2.1	Stability Studies	148
2.1.1	Descriptive Statistics	148
2.1.2	Tests of Within-Subjects Effects	149
2.1.3	Mauchly's Test of Sphericity	150
2.2	Animal Studies	150

ABSTRACT

The occurrence of resistance to chloroquine and sulfadoxine/pyrimethamine by *Plasmodium falciparum* stimulated new interest in quinine for treating multi-resistant *falciparum* infection. Parenteral quinine is the gold treatment in the management of severe and complicated malaria. There is the need for early initiation of treatment in management of complicated malaria. An antimalarial drug to be used at home must be safe, effective, affordable and easy to administer. A rectal formulation of quinine will serve the purpose of home initiation of treatment. The main objective of this work was to develop a stable quinine suppository that will ensure adequate release of quinine and evaluate quinine uptake into the four sections of the mouse brain. Cocoa butter and FattibaseTM were used in the preparation of suppositories containing 200mg quinine bisulphate. The release profiles of the formulations with varying concentrations of polysorbate 80 (0, 1, 2 and 5%) were evaluated by in-vitro dissolution in pH 8 buffer medium. Evaluation of brain uptake was carried out in various stages using the murine mice model. Quantification of uptake into the four brain sections was done with a High Pressure Liquid Chromatography technique. Uptake was compared in the four brain sections of parasitized and non-parasitized murine mice as a function of time (30, 60, 120, 180, 240 min). Quinine uptake from suppository was also compared with uptake from peritoneal injection in parasitized and non-parasitized. The values obtained were subjected to statistical analysis using the 3-way ANOVA.

The Formulations of suppositories in cocoa butter and FattibaseTM released quinine in adequate quantity. Addition of polysorbate 80 improved release of quinine significantly ($P = 0.005$ for cocoa butter and $P = 0.003$ for FattibaseTM). Cocoa butter with 1% Polysorbate 80 released 36.8% quinine bisulphate in 60 min while release from suppositories with 2% and 5% surfactant was erratic. FattibaseTM suppositories with 5% polysorbate 80 released 85% quinine content in 60min. This formulation was stable in the refrigerator for three months while samples stored at

ambient temperature were stable for one month. From the release profiles, three formulations have very high potentials in management of cerebral malaria: cocoa butter+1%, FattibaseTM + 2% and 5% respectively.

Fluorescence microscopy revealed green fluorescence characteristic of quinine in the brain sections of parasitized and non-parasitized mice treated with quinine. Quinine crossed the blood brain barrier into the brain in parasitized and non-parasitized mice. This confirms that inflammation is not required for the transport of quinine bisulphate into brain. Quinine from the suppository was available in the brain in 30 min. Uptake had a significant time-dependence ($P=0.000$). Uptake in parasitized mice was significantly higher than that in the non-parasitized mice (0.000). Quinine uptake varied significantly in the four brain sections with olfactory lobe recording the highest uptake in the two groups of mice (0.000). Quinine uptake in the parasitized mice is biphasic while a steady decline was observed in non-parasitized mice over the time period. The concentration of quinine taken up by other brain sections: cerebrum, cerebellum and medulla oblongata was significantly lower than the concentration in the olfactory lobe with cerebrum having the lowest uptake in the parasitized mice.

This intra-rectal formulation will be useful in pre-referral management procedures in primary health facilities, homes and rural areas.